

REMARKS/ARGUMENTS

The claims are 11 and 22-28. Reconsideration is expressly requested.

As an initial matter, Applicants respectfully traverse the Examiner's position that amended claim 11 is not consonant with Applicants' election of Group I, claims 2-4, 7 and 11-13 drawn to a method for the treatment of ophthalmic diseases comprising the intravenous or topical ocular administration comprising solid lipidic nanoparticles containing a pharmalogically active substance, and of triamcinolone as the species of the drug to be administered. Applicants' claims are directed to such method, and it is respectfully submitted that the Examiner's imposition of an additional restriction with respect to these claims is unwarranted. See also Applicants' Amendment filed July 10, 2009.

Claims 11, 22-24 and 27-28 were rejected under 35 U.S.C. 102(a) as being anticipated by *Cavalli et al. (Solid lipid nanoparticles (SLN) as ocular delivery system for tobramycin)*. Claims 11 and 22-28 were also rejected under 35 U.S.C. 102(b) as being anticipated by *Amselem et al. U.S. Patent No. 5,662,932*. The remaining claims were rejected under 35 U.S.C. 103(a) as

being unpatentable over *Cavalli et al.* alone (claim 25) or further in view of *Schwartz U.S. Patent No. 4,904,649* (claim 26).

This rejection is respectfully traversed.

Applicants' Amendment filed May 6, 2010 already pointed out the differences between Applicants' method as set forth in the claims and the prior art relied on by the Examiner. In addition, Applicants respectfully request the Examiner to consider the following additional comments.

Rejections based on *Cavalli et al.*

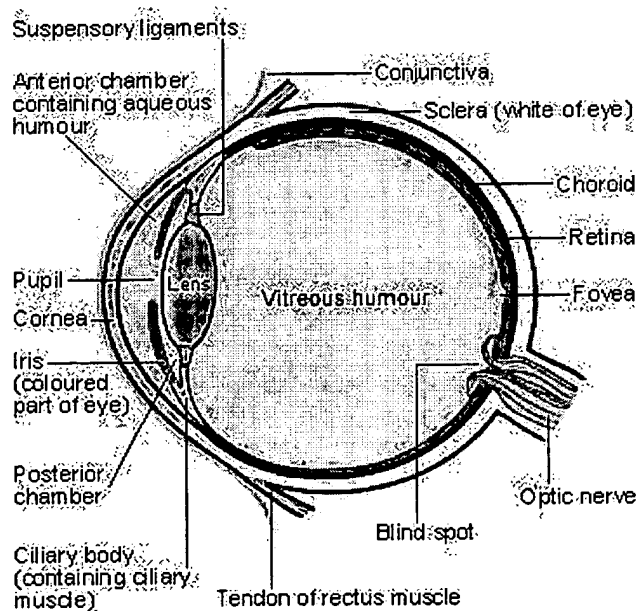
In the outstanding Office Action, with reference to *Cavalli et al.* the Examiner affirms that:

Cavalli et al. teaches the use of solid lipid nanoparticles with tobramycin topically to the eye and expressly addresses its use against bacterial endophthalmitis which would be immediately envisioned. The particles had tobramycin at 2.5%w/w, stearic acid, an average particle size of 80nm, and 0.3mg was administered to each eye in rabbits weighing 2.8-3.5kg (suspension contained 0.3%w/v TOB).

All the critical elements are taught by the cited reference and thus the claims are anticipated.

Therefore, it is respectfully submitted that the Examiner has misinterpreted the true and actual teaching of this prior art document in order to easily but improperly match the claimed invention. As a matter of fact, as already explained in Applicants' previous responses, *Cavalli et al.* expressly and unambiguously involve only the anterior fragment of the eye, since the deepest treated portion is the aqueous humour.

As clearly showed in the following section view of a human eye, the aqueous humour is a thick watery substance filling the space between the cornea and the lens:



As expressly reported both in the abstract and on page 244 (see also Fig. 1 and Table 3):

"The preocular retention of SLN in rabbit eyes was tested using drug-free, fluorescent SLN (F-SLN): these were retained for longer times on the corneal surface and in the conjunctival sac when compared with an aqueous fluorescent solution. A suspension of TOB-loaded SLN (TOB-SLN) containing 0.3% w/v TOB was administered topically to rabbits, and the aqueous humour concentration of TOB was determined up to six hours. When compared with an equal dose of TOB administered by standard commercial eyedrops, TOB-SLN produced a significantly higher TOB bioavailability in the aqueous humour." [emphasis added]

Furthermore, on page 245, it is concluded that:

"In conclusion, SLN appear as a promising vehicle for topical ocular administration of tobramycin. Their use might replace with advantage subconjunctival injections, which are necessary for treatment of 'resistant' pseudomonal keratitis, or for prophylaxis against bacterial endophthalmitis, before cataract surgery (Furigiuele et al., 1978; Ellis and Riegel, 1988)." [emphasis added]

Therefore, the teaching of Cavalli et al. as a whole is that TOB-SLN give an increased bioavailability of tobramycin in the aqueous humour that is considered promising (not proved) for

treating '**resistant**' **pseudomonal keratitis** or for **prophylaxis** against bacterial endophthalmitis **before cataract surgery**.

It should be noted that **keratitis** is a condition in which the eye's cornea, the front part of the eye, becomes inflamed.

Similarly, it should be noted that "**prophylaxis before cataract surgery**" undoubtedly is the prevention measures to be adopted in order to avoid infection post-surgery. This is confirmed by the document cited by the Examiner herself, i.e. "Callegan et al.," where on page 111, right column (the same indicated in the Office Action), it is stated:

"Infectious agents generally gain access to the posterior segment of the eye following one of three routes: (i) as a consequence of intraocular surgery (postoperative), (ii) following a penetrating injury of the globe (posttraumatic), or (iii) from hematogenous spread of bacteria to the eye from a distant anatomical site (endogenous)." [emphasis added]

In the subsequent paragraph titled "Postoperative Endophthalmitis", the authors explain that the latter "occurs most frequently following cataract surgery - the most commonly performed type of ocular surgery."

In view of that, *Cavalli et al.* only mentioned the possibility, not the certainty, of pre-treating with the antibiotic in the form of TOB-SLN in order to **prevent the penetration of infection agents before cataract surgery**, that can develop bacterial infections.

Therefore, it is respectfully submitted that the Examiner's finding that:

Cavalli et al. teaches the use of solid lipid nanoparticles with tobramycin topically to the eye and expressly addresses its use against bacterial endophthalmitis

is absolutely incorrect and against the actual understanding of the authors, who indeed only and at most **suppose** the use of TOB-SLN for **treating 'resistant' pseudomonal keratitis and prevent the onset of bacterial infections post-surgery**, supposedly in view of the pre-ocular retention of TOB-SLN in the anterior fragment of the eye, i.e. from corneal surface to aqueous humour.

It undeniably follows that *Cavalli et al.* fail to cite, and even more so concern, the therapeutically effective treatment the ophthalmic diseases specifically affecting the posterior part of

the eye, which is the most important critical element of the claimed invention.

Consequently, all the rejections based on *Cavalli et al.* should be withdrawn.

Rejections based on *Amselem et al.*

In the outstanding Office Action, with reference to *Amselem et al.* the Examiner affirms that (pages 4-5):

Amselem et al. teaches pharmaceutical composition comprising emulsomes with a lipid core including solid lipid cores. The particles have a average particle size with preferred range of 10-250nm and in certain preparations the average will fall in the range of 50-150nm. The particles (emulsomes) can be administered in several ways including topically and intravenously. A particular mode of administration described in instillation into the eye and that these compositions are similar to those of parenteral solutions. Several drugs are taught to be used with the particles including beta-adrenergic blockers (e.g. adaprolol and timolol) for glaucoma, cannibinoids, antifungal, antibiotics, corticosteroids, AIDS drugs. The lipid for the core includes triglycerides such as fatty acids in the C10-C18 range, tricaprin, trilaurin, trimyristin, tripalmitin, and tristearin. Other components that can be included are cholesterol and phospholipids.

and later on (page 5):

Example 17 also depicts the topical administration of the emulsomes comprising adaprolol maleate, tricaprin, cholesterol, and oleic acid for intraocular pressure (glaucoma) to the eye with a significant reduction of the IOP, wherein timolol would also be immediate envisioned as is known in the art as taught by Amselem. Example 20 depicts IV administration of HU-211 cannabinoid (known for anti-glaucoma) to rats at 5mg/kg wherein it would inherently affect any glaucoma present (Abstract, Col. 3 line 48-Col. 5 line 57, Col. 6 line 65-Col. 7 line 10, Col. 8 line 20-26, Col. 8 line 58- Col. 9 line 48, Col. 10 line 7-Col. 11 line 50, Examples, claims).

On page 11 of the Office Action, the Examiner also states:

Amselem teaches a solid lipid nanoparticle for ocular administration

In order to definitely clarify that the current invention makes use of Solid Lipid Nanoparticles obtained by a specific process, whereas *Amselem et al.* refer to emulsomes, Applicants herewith enclose a publication of Bhatt et al., "Lipid technology - a promising drug delivery system for poorly water soluble drugs", International Journal of Pharmaceutical Research and

Development, Sept. 2010, Vol. 2, Issue 7 (**Annex A**), where on page 2 different lipid systems for drug delivery have been identified:

- 1) Oil based formulation
- 2) Triglycerides
- 3) Liposomes and proliposomes
- 4) Niosomes
- 5) Lipid Emulsions
 - a) Simple emulsions :o/w emulsion, w/o emulsion
 - b) Multiple emulsions: o/w/o emulsion, w/o/w emulsion
- 6) Emulsome
- 7) Hydrogel nanoparticles
- 8) Aquasomes
- 9) Solid solutions using PEG (Polyethylene glycol) and PVP(Ploy vinyl pyrrolidone)
- 10)Solid lipid nanoparticles
- 11)Nanostructure lipid carriers (NLC)
- 12)L-OROS Technology
- 13)SEDDS (Self emulsifying drug delivery systems) and SMEDDS (Self micro emulsifying drug delivery systems)

On page 5, emulsomes are defined as follows:

6) EMULSOME^{15,16}

Emulsome represents lipid based drug delivery systems with wide range of therapeutic applications especially for parenteral delivery of drugs which are poorly water soluble. Emulsome particles basically consist of microscopic lipid assembly with apolar core which contains water insoluble drugs in the solution form without requiring any surface active agent or co solvent. These fat cored lipid particles are dispersed in an aqueous phase. These systems are often prepared by melt expression or emulsion solvent diffusive extraction.

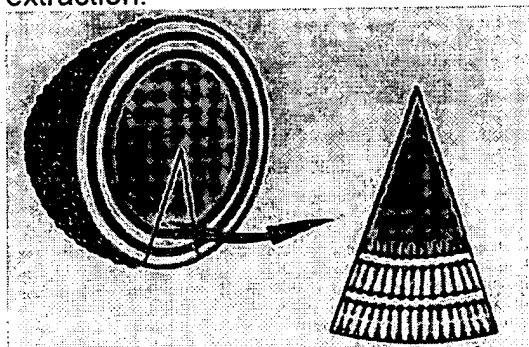


Figure 5 - Diagrammatic Structure of Emulsome

whereas on page 6, the Solid Lipid Nanoparticles (SLN) are treated:

10) SOLID LIPID NANOPARTICLES 26,27,28,29,30,31,32

They are prepared from solid lipids. They are submicron colloidal carriers (50-1000nm) which are composed generally of lipid dispersed in water or in an aqueous surfactant solution. The advantages of SLNs are:

- Their small size and relatively narrow size distribution permits site specific drug delivery.
- Controlled and sustained release of active drug can be achieved.
- The incorporated drug is protected from the onslaughts of biochemical degradation.
- Can be lyophilized.
- Relatively cheap and stable.

The lipid nanoparticles were prepared by first melting the lipid and it was dispersed in a hot aqueous surfactant by stirring or ultrasonic treatment. Micro emulsion technique was used for the preparation of solid lipid nanoparticles. The hot micro emulsion containing the lipid was poured in to cold water leading to solidification of nanoparticles. SLNs facilitate prolonged drug release and possess lower cytotoxicity.

This document demonstrates that, even to date, emulsomes and SLN are widely and well recognized different and distinct delivery systems.

It is further reminded that Amselem *et al.* make use of organic solvent, like dichloromethane and diethyl ether, in

preparing emulsomes, whereas the SLN of the current invention as recited in the claims do not involve any organic solvent at all.

Additionally, the Examiner affirms that:

glaucoma is not a disease of the optic nerve but is the condition, most commonly the high intraocular pressure, that is the cause of the deterioration of the optic nerve.

In order to definitely clarify also this point, Applicants provide an extract from Goodman & Gilman's "The pharmacological basis of therapeutics" 9th Edition (1996), as **Annex B**.

In this extract, the following pages have been selected:

- page 1625, where the "posterior segment" of the eye is defined and described;
- page 1630, where infection diseases, like Keratitis and Endophthalmitis, are discussed; and
- page 1633, where "glaucoma" is defined to be characterized by progressive optic nerve cupping and visual field loss. It should be noted that with

reference to different causes for glaucoma, it is stated:

Although particularly elevated intraocular pressures (e.g., greater than 30 mm Hg) usually will lead to optic nerve damage, certain patients' optic nerves appear to be able to tolerate intraocular pressures in the mid-to-high twenties. These patients are referred to as *ocular hypertensives*, and a prospective multicenter study is currently under way to determine whether or not early medical treatment to lower intraocular pressure will prevent glaucomatous optic nerve damage. Other patients have progressive glaucomatous optic nerve damage despite having intraocular pressures in the so-called normal range, and this form of the disease is sometimes called *normal tension* or *low tension* glaucoma.

In the enclosed **Annex C**, a Normal-Tension Glaucoma overview is given, in order to explain that an absolutely non negligible percentage of glaucoma patients do not show high IntraOcular Pressure (IOP).

For sake of completeness, a glaucoma overview from Wikipedia has been provided, as **Annex D**, where it is affirmed that "Glaucoma is an eye disorder in which the optic nerve suffers damage, permanently impacting vision in the affected eye(s) and progressing to complete blindness if untreated. It is often, but

not always, associated with increased pressure of the fluid in the eye (aqueous humour). The nerve damage involves loss of retinal ganglion cells in a characteristic pattern."

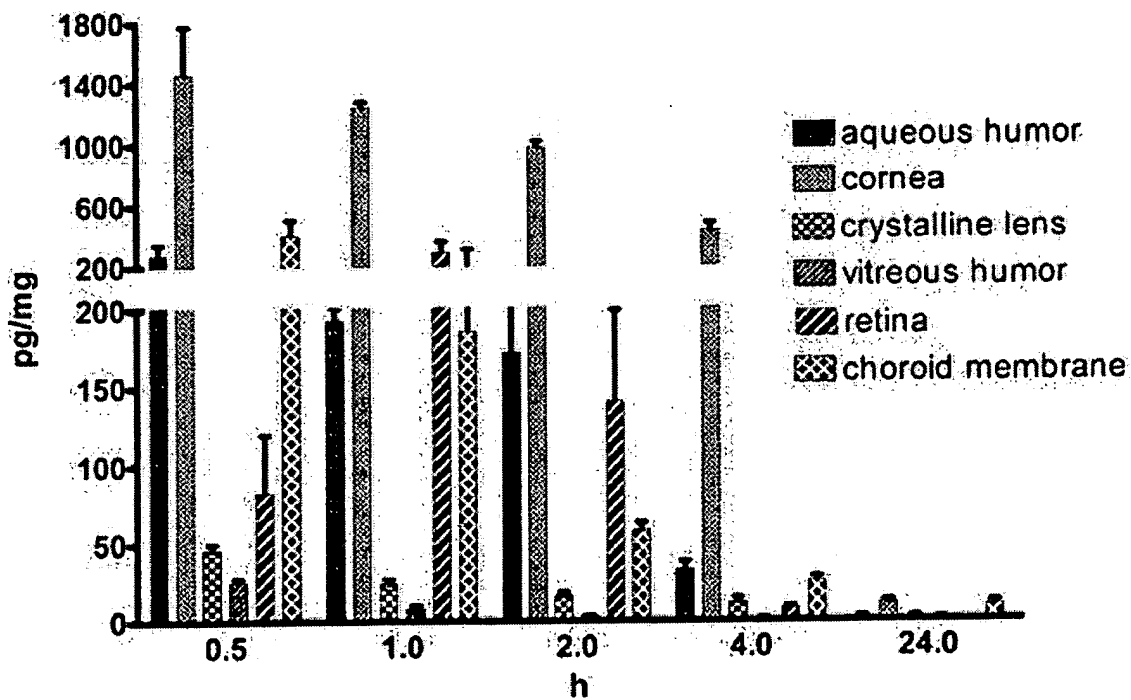
Therefore, emulsomes of *Amselem et al.* are only deemed to intervene on the IOP by topically treating the anterior segment of the eye, specifically the aqueous humour, achieving at most the technical effect of lowering the Intra-Ocular Pressure (IOP), which is only one of the risk factors for glaucoma (as stated also in http://en.wikipedia.org/wiki/Intraocular_pressure). Therefore, also in this case, at most a prevention method is supposed for glaucoma, but not a method of treatment after the onset of the disease.

Finally, **Annex E** is an abstract relating to a study of Viola et al., "Solid Lipid Nanoparticles topically administered in rabbits as new drug delivery system: a preliminary study of safety and bioavailability", ARVO 2009 annual meeting, Fort Lauderdale, 2009.

This study further demonstrates that the SLN of the current invention as recited in the claims, in this case carrying Triamcinolone acetonide as drug, achieved **in a surprising amount**

as well as in a surprising short time also the choroid membrane, which is the vascular layer of tissue between the retina and the sclera of the eye. The macula responsible for central vision and the anterior part of the optic nerve are dependent on choroidal blood supply.

The results of this study are represented below:



It undeniably follows that Amselem et al. fail to make use of SLN as defined in Claim 11 and fail to cite, let alone concern, the therapeutically effective treatment of the posterior part of the eye, which is indeed achieved by the claimed method.

Consequently, also all the rejections based on *Amselem et al.* should be withdrawn.

Applicants wish to add that the Examiner appears to have misunderstood the current invention as recited in the claims with respect to a further aspect when she affirms at page 12 that:

Even attempts by others after

the instant filing date address that there are no drugs that yet been shown to protect retinal ganglion cells (see Woodward et al.).

It is respectfully submitted that the current invention as recited in the claims does not address at all the search of new drugs, but rather refers to a **surprising method for carrying drugs to the posterior segment of the eye so that the drugs successfully act there**, where previously only surgical operations were envisaged.

Furthermore, the Examiner alleged that what discussed in the Declaration of Prof. M. R. Gasco submitted with Applicants' May 6, 2010 Amendment could not be considered, since retinitis pigmentosa is not present in the claims. In this regard, the Applicants enclose herewith also **Annex F**, where Franceschetti et al. dealt with viral retinitis pigmentosa. Thus, in view of the fact that "viral retinitis" is listed in Claim 11, the data discussed in the Declaration should be fully considered and found persuasive.

In fact, while the studies further confirmed the efficacy of the claimed method, at the same time, these results are even more surprising when considering that **no prior art at all has been found by the Examiner addressing the treatment of the posterior segment of the eye, thus preventing the skilled person from even considering this possibility.** Additionally, Applicants wish to remark that the only alternative way to reach the retina was intravitreal injections that are extremely painful and extremely tricky in terms of risks of injuring the eye and promoting infections. Therefore, since the injections are definitely undesirable and unsatisfactory under many points of view, the contribution of the claimed invention over the prior art is even more evident and appreciable.

In order to strengthen the Applicants' position, the following documents are also enclosed:

Annex H: Stretto et al. "Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of retinitis pigmentosa"; and

Annex I: Stretto et al. 10.1073/pnas.1007644107 (Supporting Information).

The documents further demonstrate that the SLN of the current invention successfully reach the retina, since (see abstract):

"Noninvasive treatment was achieved using eye drops consisting of a suspension of solid lipid nanoparticles loaded with myriocin. Short-term noninvasive treatment lowered retinal ceramide in a manner similar to intraocular injections, indicating that nanoparticles functioned as a vector permitting transcorneal drug administration. Prolonged treatment (10-20 d) with solid lipid nanoparticles increased photoreceptor survival, preserved photoreceptor morphology, and extended the ability of the retina to respond to light as assessed by electroretinography. In conclusion, pharmacological targeting of ceramide biosynthesis slowed the progression of RP in a mouse model, and therefore may represent a

therapeutic approach to treating this disease in humans. Transcorneal administration of drugs carried in solid lipid nanoparticles, as experimented in this study, may facilitate continuous, noninvasive treatment of patients with RP and other retinal pathologies."

As far as the non-obviousness of the claimed invention is concerned, in addition to what above argued, Applicants maintain the position that Cavalli et al. clearly does not pertain to the field of endeavour of the present invention and that nothing in the document discloses or suggests Applicants' invention as recited in the claims. Not only does *Cavalli et al.* fail to suggest at all the method for treating the posterior segment of the eye, but also *Cavalli et al.* does not call for any expectation of success, because the TOB-SLN was taught to reach only the aqueous humour.

The Examiner refers also to a further document cited in the introduction of *Cavalli et al.*, i.e. *Cavalli et al.* (1995) "Preparation and evaluation in vitro of colloidal lipospheres containing pilocarpine as ion pair" (**Annex G**).

It should be noted that not only the lipospheres were not prepared under the process conditions of Claim 11, but also merely their stability and in vitro drug release have been

reported, thus leaving totally uninvestigated all the other aspects. In fact, the authors concluded at page 245, generally affirming that the lipospheres can be a promising sustained-release ocular formulation.

This further means and confirms that **at the time the invention was made, the skilled person actually had no pertinent background art where to start from**.

As a matter of fact, **there is no antecedent** for a method of treating specific ophthalmic diseases of the posterior segment of the eye through intravenous or topical ocular administration of a therapeutically effective amount of a pharmacologically active substance suitable for the treatment of the ophthalmic diseases, the active substance being incorporated into solid lipidic nanoparticles obtained by a specific process.

Applicants also provide a **Declaration of Dr. Gian Paolo Zara**, who is one of the inventors, wherein comparative Examples have been discussed between the method of the invention and known methods in the art and a further comment against Amselem has been provided.

PROVISIONAL DOUBLE PATENTING OBJECTION

Claims 11 and 22-28 were provisionally rejected on the grounds of non-statutory obviousness-type double patenting as being unpatentable over claims 89 and 91 of co-pending U.S. Patent Application Serial No. 11/629,141.

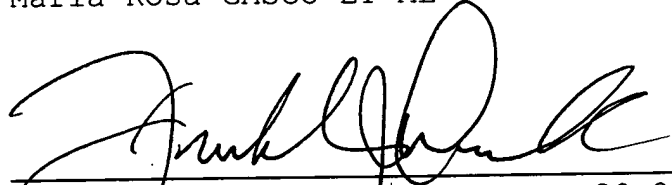
In view of this reiterated objection, Applicants reserve the right to cancel Claims 89 and 91 of the copending US application No. 11/629,141, once the US patent has been granted for the current US application.

CONCLUSIONS

In view of the above, Applicants submit that the Application as currently pending is in condition for allowance on the grounds that the amendments provided in Applicants' Amendment filed May 6, 2010 fully overcome the objections raised in the outstanding Office Action.

Applicants also submit herewith a Third Supplemental
Information Disclosure Statement.

Respectfully submitted,
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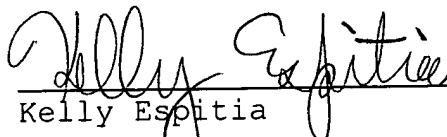
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Enclosures:

Annexes A-I

Declaration under 37 C.F.R. 1.132 of Dr. Gian Paolo Zara
Third Supplemental Information Disclosure Statement
Form PTO-1449 with twenty-one (21) documents
Check in the amount of \$180.00

I hereby certify that this correspondence is being deposited
with the U.S. Postal Service as first class mail in an envelope
addressed to: MAIL STOP AMENDMENT, Commissioner for Patents, P.O.
Box 1450, Alexandria, VA 22313-1450, on February 2, 2011.



Kelly Espitia

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ANNEX A

LIPID TECHNOLOGY- A PROMISING DRUG DELIVERY SYSTEM FOR POORLY WATER SOLUBLE DRUGS



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ABSTRACT

Today a huge problem of poorly water soluble drugs is being faced in the world. Many methods are there for increasing the solubility, but lipid technology is one of the most prominent and latest technology. Lipid formulations always produce a fine dispersion which is useful for increasing the solubility of poorly water soluble drugs. So the main objective is to increase the solubility by various techniques related to lipid technology. The study methodology includes different formulations which are as follows: Oil based formulations, Triglyceride, liposomes, niosomes, lipid emulsions, emulsome, hydrogel nanoparticles, aquasomes, solid lipid nanoparticles, nanostructure lipid carriers.

Key Words: poorly water soluble, lipid technology, dispersion, liposomes, aquasomes, solid lipid nanoparticles

INTRODUCTION

Bioavailability is defined as the rate and the extent to which the ingredients or active moiety is absorbed from the drug product and becomes available at the site of action. As per the definition of bioavailability, a drug with poor bioavailability is one with poor aqueous solubility, slow dissolution rate in biological fluids, poor stability of dissolved drug at physiological pH, poor permeation through biomembrane, extensive presystemic

metabolism. Bioavailability of poorly water soluble drugs is a major problem. There are three major approaches to overcome the bioavailability problems.

A) Pharmaceuticals approach:

Modification of formulation, manufacturing processes or physiochemical properties of the drug is done.

B) Pharmacokinetic approach:

Pharmacokinetics of drug is altered by modifying its chemical structure.

C) Biological approach:

In this, route of drug administration may be changed such as parenteral form instead of oral form. Rate dissolution and its solubility are very important factors in third approach. The second approach of chemical modification has number of drawbacks such as being very expensive, time consuming, requires repetition of chemical studies, risk of precipitation and adverse effects. So generally only pharmaceuticals approach is considered there.

The technology which has the potential to solubilise varying quantities of poorly water soluble drugs with the help of lipids or lipid systems is known as lipid technology.

Various lipid systems are as follows:

- 1) Oil based formulation
- 2) Triglycerides
- 3) Liposomes and proliposomes
- 4) Niosomes
- 5) Lipid Emulsions
 - a) Simple emulsions :o/w emulsion, w/o emulsion
 - b) Multiple emulsions: o/w/o emulsion, w/o/w emulsion
- 6) Emulsome
- 7) Hydrogel nanoparticles
- 8) Aquasomes
- 9) Solid solutions using PEG (Polyethylene glycol) and PVP(Poly vinyl pyrrolidone)
- 10) Solid lipid nanoparticles
- 11) Nanostructure lipid carriers (NLC)
- 12) L-OROS Technology
- 13) SEDDS (Self emulsifying drug delivery systems) and SMEDDS (Self micro emulsifying drug delivery systems)

Lipid formulations always produce a fine dispersion of poorly water soluble drugs. Many lipid systems are colloidal in nature. Lipids can reduce the inherent limitations of slow and incomplete dissolution of poorly water soluble drugs and facilitates formation of solubilised

phases from which drug absorption occurs. In any vehicle mediated delivery (whether the vehicle is an emulsion, micro particle, liposome, niosome or other lipid system) not only is the rate and mode of drug release from the system important, but it is important in relation to the rate of movement of delivery system.

1) OIL BASED FORMULATIONS ¹

Lipids are ideally prepared as unit dosage forms such as sealed hard or soft gelatin capsules. Oils may be used as vehicles for oral drug delivery. In this case, the volume of oil given is low, typically less than 0.5ml per capsule. Oil based formulations offer many advantages in the formulation of poorly water soluble drugs. The soft gelatin capsule is generally perceived by the consumer as an elegant, easy to swallow dosage form but this type of formulation is not widely employed due to interaction of the fill with soft gelatin shell, limited solubility of some drugs in the lipid solvents, need to incorporate suspending agents for poorly water soluble drugs, stability and manufacturing methods and a lack of knowledge of what happens to the dosage form in vivo. In drug absorption studies, the administration of drugs with a fatty meal results in a marked delay in the onset of the absorption phase. The delay is extremely marked for enteric coated dosage forms which may be retained in the stomach for more than six hours. Arachis oil, castor oil, cotton seed oil, maize oil, olive oil, sesame oil, sunflower oil are the various lipophilic liquid vehicles. E.g. Halofantrine hydrochloride (Hf.HCl) is a new important antimalarial water insoluble drug. There was a 3- fold increase in the mean oral bioavailability of 250mg Hf.HCl when administered with a fatty meal to human subjects.

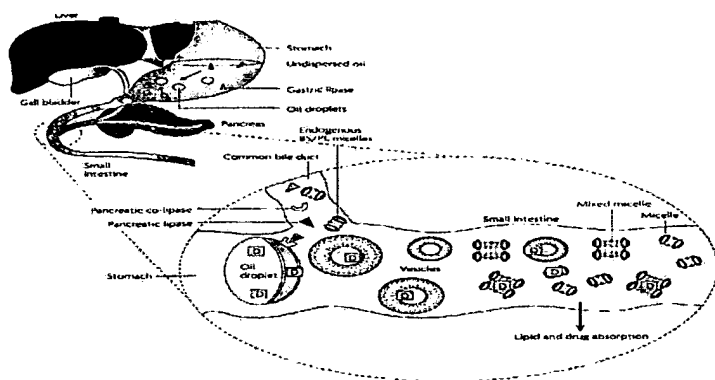


Figure 1 – Pathway showing the metabolism of oil based formulations

2) TRIGLYCERIDES^{2,3}

Triolin is a triglyceride of oleic acid and representative of the major constituent in vegetable oils such as soyabean oil. Such oils are one of the key ingredients in lipid emulsions such as Intralipid, a parenteral emulsion employed for solubilization of insoluble drugs. MCT (Medium Chained Triglycerides) such as tricaprylin may be considered for both oral and intravenous lipid formulations. MCT are digested more rapidly which may improve oral bioavailability. The potential for significant concentrations of water to exist in the lipid phase may be important factor to consider for medium chain triglycerides or other lipid vehicles that contain relatively high ester bond or other polar functional group concentrations. Softisan 645, Akomed E, Labrafac CC, Miglyol 810, Lauroglycol FCC are the various lipophilic liquid vehicles.

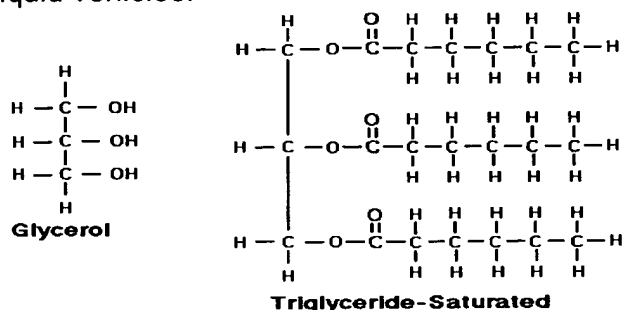


Figure 2 - Chemical Structure of Triglyceride

3) LIPOSOMES⁴

Liposomes were discovered in the early 1960's by Bangham and colleagues and subsequently became the most extensively explored drug delivery system. Structurally liposomes are concentric bilayered vesicles in which an aqueous volume is entirely enclosed by a membranous lipid bilayer mainly composed of natural or synthetic phospholipids. Liposomes are formed when phospholipids are hydrated. The most common natural phospholipids are phosphatidylcholine (PC). These are amphiphilic molecules in which a glycerol bridge links to a pair of hydrophobic acyl hydrocarbon chains with a hydrophilic polar head group phosphocholine. Amphiphilic nature of phospholipids and their analogues render them the ability to form closed concentric bilayers in the presence of water. Liposomes are formed when thin films of amphiphilic nature are hydrated and stacks of liquid crystalline bilayers become fluid and swell. The hydrated lipid sheets detach during agitation and self close to form large multilamellar vesicles (MLVs). Sonification is done to get small unilamellar vesicles (SUVs). Extrusion is also done to get large unilamellar vesicles (LUVs). Several methods exist for improved loading of drugs using pH gradients and potential difference across liposomal membranes. The pH gradient is created by preparing liposomes with a low pH inside the vesicles followed by the addition of the base to the extra liposomal medium. Accumulation occurs at the low pH side. So the unprotonated form of basic drug can diffuse through the bilayer. At the low pH side, the molecules are predominately protonated which lowers the concentration of drug in the unprotonated form and thus promotes the diffusion of more molecules at the low pH side of the bilayer. Stealth liposomal technology is designed for the intravenous drug delivery.

Proliposomes⁵:

In order to increase the surface area of dried lipid film and to facilitate hydration, the lipid is dried over a finely divided particulate support. These dried lipid coated particulates are called pro-liposomes. Pro-liposomes form dispersion of MLVs on adding water into them. This method overcomes the stability problems of liposomes encountered during their storage as dispersion, dry or frozen form. It is ideally suited for preparations where the drug to be entrapped incorporated into lipid membrane.

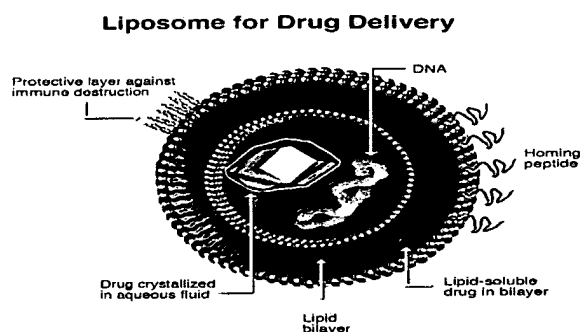


Figure 3 – Structure of Liposome

4) NIOSOMES^{6,7,8}

Non ionic surfactant vesicles (Niosomes or NSVs) are now widely studied as an alternative to liposomes. Non-ionic surfactant vesicle results from self assembly of hydrated surfactant monomers. Niosomes are essentially non ionic surfactant based multi or unilamellar vesicles in which an aqueous solution is entirely enclosed by a membrane resulted from the organization of surfactant macromolecules as bilayers. Ether injection, hand shaking method, sonification, reverse phase evaporation, aqueous dispersion and extrusion are various methods of preparation of niosomes.

5) LIPID EMULSIONS^{9,10}

Emulsions are heterogeneous systems in which one immiscible liquid is dispersed as droplets in

another liquid. Lipid emulsions are suitable for both passive and active drug targeting. Sometimes size specific delivery can be designed by attaching globules. Lipid emulsion drug delivery systems seem to offer a wide variety of possibilities for preparing better tolerated intravenous formulations of poorly water soluble drugs while either maintaining the same characteristics engineered to pharmacokinetic and tissue distribution or enhancing site specific delivery to target organs. E.g. Intralipid is the first approved intravenous lipid emulsion for parenteral nutrition and consists of an o/w emulsion of 10 or 20% soyabean oil droplets (70-400nm size) stabilized by monolayer of egg yolk mixed phospholipids (1.2%) and glycerol (2.25%) as an osmotic agent. They are of 2 types

- Simple emulsions :o/w emulsion (dispersion of oil droplets in aqueous phase), w/o emulsion (dispersion of droplets of aqueous phase in oil phase)
- Multiple emulsions: o/w/o emulsion, w/o/w emulsion
- Micro emulsions^{11,12}.

Micro emulsions are isotropic and thermodynamically stable multicomponent fluids composed of water, oil, surfactant and co-surfactant. They optimize the performance of a wide spectrum of products and processes. The diameter of droplets in a micro emulsion is in the range of 100Å-1000Å where as the diameter of droplets in a kinetically stable macro emulsion is 50 micrometer. This includes normal micellar solutions, reverse micelles, cores or droplets of water or oil and for some systems, even bicontinuous structures in which neither oil nor water surrounds the other.

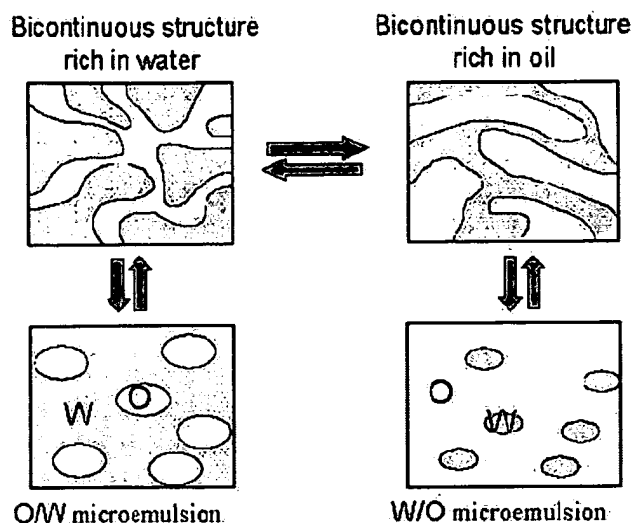


Figure 4 – Diagrammatic Structure of two types of microemulsion

Sub micron emulsions¹³:

Sub micron lipid emulsions are potential drug carriers for lipophilic and amphiphilic drugs with many favorable properties. They are biocompatible, biodegradable, stable and easy to prepare and handle. The basic structure is a neutral lipid core (i.e. triglyceride) stabilized by a monolayer of amphiphilic lipid (i.e. phospholipid). Such emulsions can solubilize considerable amounts of lipophilic drugs in the core and amphiphilic drugs in the surface monolayer. Emulsification can be performed by applying brief sonification or by pressure homogenization. Drugs for effective incorporation into lipid emulsions should be preferably oil soluble or amphiphilic.

Multiple emulsions¹⁴:

Multiple emulsions are complex systems and they are called emulsions of emulsions, double or triple emulsions because of the internal phase containing dispersed globules which are miscible with the continuous phase. This leads to w/o/w or o/w/o type where the two miscible phases are separated by an immiscible phase. This phase is called a liquid membrane which acts a semi permeable membrane through

which a solute may diffuse from one phase to another. Thus multiple emulsions are also called as liquid membrane systems. These systems are characterized by their low thermodynamic stability. In most cases, the two aqueous phases are identical and therefore a w1/o/w1 emulsion is a second order two component system and an o1/w/o2 emulsion is a three component second order system. Emulsion can be prepared by remicellization of one dispersed phase into another continuous phase. The intent in preparing multiple emulsion systems is to introduce two different surfactants of opposite nature to the system. One surfactant stabilizes the w/o emulsions while the other stabilized the o/w emulsion,

6) EMULSOME^{15,16}

Emulsome represents lipid based drug delivery systems with wide range of therapeutic applications especially for parenteral delivery of drugs which are poorly water soluble. Emulsome particles basically consist of microscopic lipid assembly with apolar core which contains water insoluble drugs in the solution form without requiring any surface active agent or co solvent. These fat cored lipid particles are dispersed in an aqueous phase. These systems are often prepared by melt expression or emulsion solvent diffusive extraction.

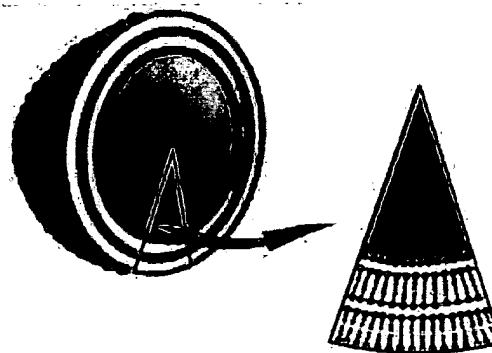


Figure 5 - Diagrammatic Structure of Emulsome

7) HYDROGEL NANOPARTICLES ^{17,18,19,20}

The self assemblage and self aggregation of natural polymer amphiphiles such as hydrophobized polysaccharides in water produces hydrogel nanoparticles. The cholesterol bearing polysaccharides (CHP) self aggregate to form monodisperse and stable hydrogel nanoparticles. In these nanoparticles, domains of the associated cholesterol groups of cholesteryl polysaccharides offer non covalent cross linking points which helps in increasing the solubility of poorly water soluble drugs.

8) AQUASOMES ²¹

Aquasomes are three layered self assembling compositions with ceramic carbon nanocrystalline particulate core coated with glassy cellobiose or alternatively degradable calcium phosphate nanocrystalline particle core coated with glassy pyridoxal-5 phosphate. Here, drug is covalently bound to the outer coating. These complex multicomponent particulate delivery systems are assemblies of simple polymers, complex lipid mixtures or ceramic materials with diameter ranging between 30 to 500 nm. Aquasomes deliver their contents through a combination of specific targeting, molecular shielding and a slow sustained release processes. Their large sized and active surfaces enable them to be loaded with water insoluble drugs through non covalent processes.

9) SOLID SOLUTIONS USING PVP AND PEG ^{22,23,24,25}

Lipids are mixed with nanoparticles in apolar solvent. Mixed film is hydrated and thereafter separated by centrifugation. This is the encapsulating procedure of hydrophobic nanoparticles in micelles. Another strategy for poorly soluble drugs is to formulate a solid solution using a water soluble polymer to aid

solubility of the drug compound. E.g. polyvinyl pyrrolidone (PVP) and polyethylene glycol (PEG 6000) have been used for preparing solid solutions with poorly soluble drugs. Solid solutions can be prepared by dissolving both the drug compound and the polymer in a suitable volatile solvent. On removing the solvent (fluid bed drying), an amorphous drug-polymer is produced. Alternatively, it is sometimes possible to dissolve the drug directly in the molten polymer. On cooling, the drug is then trapped in an amorphous state within the water soluble polymer matrix, thus enhancing the water solubility of the drug.

10) SOLID LIPID NANOPARTICLES ^{26,27,28,29,30,31,32}

They are prepared from solid lipids. They are submicron colloidal carriers (50-1000nm) which are composed generally of lipid dispersed in water or in an aqueous surfactant solution. The advantages of SLNs are:

- Their small size and relatively narrow size distribution permits site specific drug delivery.
- Controlled and sustained release of active drug can be achieved.
- The incorporated drug is protected from the onslaughts of biochemical degradation.
- Can be lyophilized.
- Relatively cheap and stable.

The lipid nanoparticles were prepared by first melting the lipid and it was dispersed in a hot aqueous surfactant by stirring or ultrasonic treatment. Micro emulsion technique was used for the preparation of solid lipid nanoparticles. The hot micro emulsion containing the lipid was poured in to cold water leading to solidification of nanoparticles. SLNs facilitate prolonged drug release and possess lower cytotoxicity.

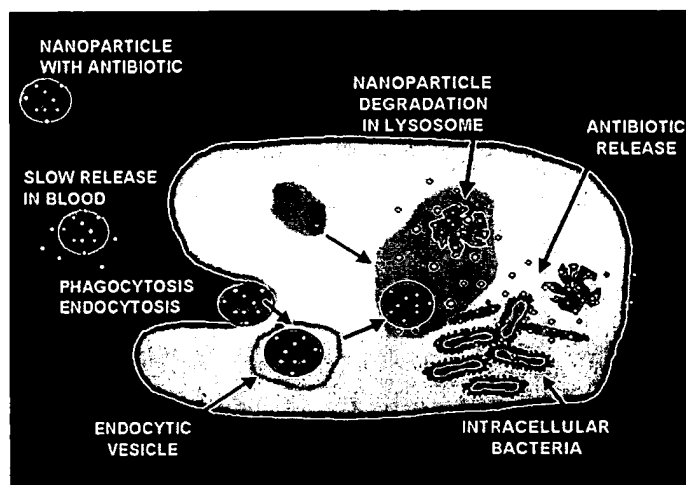


Figure 6 – Action of Solid Lipid Nanoparticles

11)NLC(NANOSTRUCTURE LIPID CARRIERS)^{33,34,35}

They are the nanostructure lipid dispersions with solid contents produced by high pressure homogenization. Lipid drug conjugate provide high loading capacity for hydrophilic drugs for oral delivery which in turns help to increase the solubility of poorly water soluble drugs.

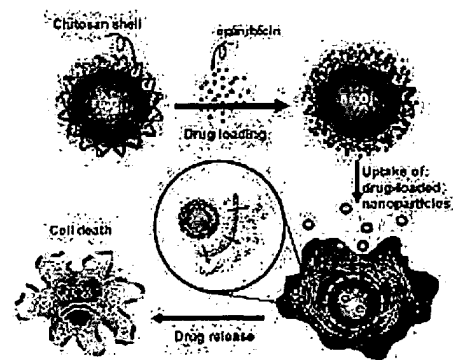


Figure 7 – Action of NLC

12)L-OROS TECHNOLOGY^{36,37}

ALZA developed the L-OROS system, an OROS delivery system adapted for poorly water soluble drugs. These formulations include self emulsifying liquid carrier formulations (SEF) that

allow a drug to be more readily absorbed through gastro intestinal membrane and blood stream. The SEF in L-OROS systems consists of drugs in non aqueous liquid carriers formulated to give either a solution or a nanosuspension. As drug in solution is released in GI tract, it forms very small droplets (<100nm), increasing the drugs solubility, thereby enhancing bioavailability. As the drug in a nanosuspension is released, the drug nano particles are dispersed and aggregation is prevented.

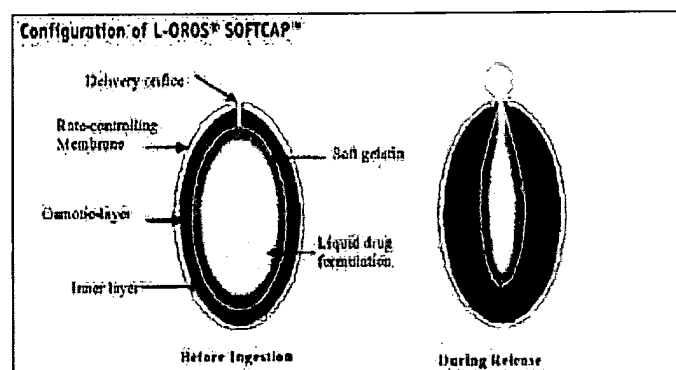


Figure 8 – Schematic Diagram showing Drug release

13)SEDDS (SELF EMULSIFYING DRUG DELIVERY SYSTEM)^{38,39,40,41} AND SMEDDS(SELF MICRO EMULSIFYING DRUG DELIVERY SYSTEM)^{42,43,44,45,46}

Recent developments in formulation science have included the use of mixtures of lipid excipients and surfactants to produce self emulsifying drug delivery systems (SEDDS) and self micro emulsifying drug delivery systems (SMEDDS) for oral administration of poorly water soluble drugs. These are formulations that form emulsions or micro emulsions spontaneously on contact with aqueous media. SMEDDS are distinguished from SEDDS by the smaller emulsion droplets produced on dilution, resulting in a transparent or translucent solution. SMEDDS generally contain high concentrations

of surfactant (40-60%) and regularly contain hydrophilic co solvents (e.g. propylene glycol, polyethylene glycols). They are often described as micro emulsion preconcentrates as the micro emulsion is formed on dilution in aqueous media. An example of product which uses a SMEDDS type of formulation is Neoral (Novartis) for oral administration of cyclosporine. In relation to formulation development of poorly water soluble drugs in the future, there are now techniques being used to convert liquid/semi solid SEDDS and SMEDDS formulations into powders and granules. There is also increasing interest in using inert adsorbents such as Neusilin (Fuji chemicals) and Zeopharm (Huber) products for converting liquids into powders which can then be processed into powder fill capsules or tablets. Gelucire 44/14 is reversible heat meltable excipients and has proved to be of great interest in the manufacturing of semi solid formulations. It is also commonly used as an excipient for immediate release dosage form that increases solubility of hydrophobic drugs and enhances bioavailability. Gelucire is composed of fatty acid esters of glycerol, PEG esters (mono and diesters). Another example is of Ontazolast which is a potent inhibitor of calcium ionophore. This compound is practically insoluble in water. The bioavailability of ontazolast was significantly and substantially enhanced by all of lipid based formulations.

CONCLUSION:

The major problem is bioavailability of poorly water soluble drugs especially for new molecules which are being synthesized. Thus the main target is to increase the rate of poorly water soluble drugs. These are numerous techniques for enhancement of solubility but out of these, lipid technology is reviewed in this literature survey. Some of these methods have been commercialized like liposomes, niosomes,

aquasomes, solid lipid nanoparticles, etc, but still lot of input is required from the Pharma industries and formulators to commercialize other methods also. Many drug delivery and pharmaceutical companies are exploiting this technology to reexamine active ingredients that were abandoned from formulation programs because of their poor solubility. With the tremendous increasing research and development and with the advent of new technologies and commercial success in lipid technology, it will definitely continue to appeal to both pharmaceutical researchers and the pharmaceutical industry. Lipid technology is the latest trend which has affected the Pharma field and is growing by leaps and bounds. Many methods have been patented and commercialized and the future belongs to lipid technology.

REFERENCES:

1. Shui MK, Andrew JH, Christopher JH, Glenn AE, William NC. Formulation, design and bioavailability assessment of lipidic self-emulsifying formulations of halofantrine. *Int J Pharm.*1998; 167: 155-164.
2. Joseph A, René T, Armand B, Douglas SM, Robert WL. Solubility of milk fat triglycerides in supercritical carbon dioxide. *Food Res Int.* 1994; 27: 459-467
3. Traul KA, Driedger A, Ingle DL, Nakhasi D. Review of the toxicologic properties of medium-chain triglycerides. *Food Chem Toxicol.* 2000; 38: 79-98.
4. Dan DL. Novel applications of liposomes. *Trends in Biotechnology.* 1998; 16: 307-321
5. Hee JL, Byung NA, Woo HP, Chang KS, Myung GL. Inverse targeting of reticuloendothelial system rich organs after intravenous administration of adriamycin-loaded neutral proliposomes containing poloxamer 407 to rats. *Int J Pharm.* 1996; 131: 91-96.

6. Ijeoma FU, Suresh PV. Non-ionic surfactant based vesicles (niosomes) in drug delivery. *Int J Pharm.* 1998; 172: 33-70.
7. Inas AD, Ijeoma FU. The evaluation of crown ether based niosomes as cation containing and cation sensitive drug delivery systems. *Int J Pharm.* 1997; 159: 207-213.
8. Maria M, Chiara S, Donatella V, Francesco L, Anna MF. Niosomes as carriers for tretinoin: III. A study into the in vitro cutaneous delivery of vesicle-incorporated tretinoin. *Int J Pharm.* 2006; 311: 11-19.
9. Xu J, Li YD. Formation of zinc sulfide nanorods and nanoparticles in ternary W/O microemulsion. *J Colloid Interface Sci.* 2003; 259: 275-281.
10. Zhao QT, Hou LS, Huang R. Synthesis of ZnS nanorods by a surfactant-assisted soft chemistry method. *Inorg Chem Commun.* 2003; 6: 971-973.
11. Xu S, Zhou HC, Xu J, Li YD. Synthesis of size-tunable silver iodide nanowires in reverse micelles. *Science and Technology of Advanced Materials.* 2000; 18: 10503-10504.
12. Lv RT, Cao CB, Zhu HS. Synthesis and characterization of ZnS nanowires by AOT micelle-template inducing reaction. *Mater Res Bull.* 2004; 39: 1517-1524.
13. Lv RT, Cao CB, Guo YJ, Zhu HS. Preparation of ZnS nanotubes via surfactant micelle-template inducing reaction. *J Mater Sci.* 2004; 39: 1575-1578.
14. Kim HS, Sigmund W. Zinc sulfide nanocrystals on carbon nanotubes. *J Cryst Growth.* 2003; 255: 114-118.
15. Swati G, Anuradha D, Suresh PV. Antileishmanial efficacy of amphotericin B bearing emulsomes against experimental visceral leishmaniasis. *J Drug Target.* 2007; 15: 437-444.
16. Swati G, Suresh PV. Development and characterization of amphotericin B bearing emulsomes for passive and active macrophage targeting. *J Drug Target.* 2007; 15: 206-217.
17. Mehrdad H, Amir A, Pedram R. Hydrogel nanoparticles in drug delivery. *Adv Drug Deliv Rev.* 2008; 60: 1638-1649.
18. Kun N, Kwang HL, You HB. pH-sensitivity and pH-dependent interior structural change of self-assembled hydrogel nanoparticles of pullulan acetate/oligo-sulfonamide conjugate. *J Control Release.* 2004; 97: 513-525.
19. Liandong D, Yinglei Z, Xiaona L, Fengmin J, Xiaohua H, Anjie D. Investigation on properties of re-dispersible cationic hydrogel nanoparticles. *Eur Polymer J.* 2008; 44: 978-986.
20. Sook K, Young J. Self-assembled hydrogel nanoparticles composed of dextran and poly(ethylene glycol) macromer. *Int J Pharm.* 2002; 205: 109-116.
21. Irma RO, Rodrigo AS, José RG. Elaboration and structural analysis of aquasomes loaded with Indomethacin. *Eur J Pharm Sci.* 2007; 32: 223-230.
22. Fawaz F, Bonini F, Guyot M, Bildet J, Maury M, Laguery AM. Bioavailability of norfloxacin from PEG 6000 solid dispersion and cyclodextrin inclusion complexes in rabbits. *Int J Pharm.* 1996; 132: 271-275.
23. Taboada ME, Asenjo JA, Andrews BA. Liquid-liquid and liquid-liquid-solid equilibrium in Na₂CO₃-PEG-H₂O. *Fluid Phase Equilibria.* 2001; 180: 273-280.
24. Shivakumar GK, James WA. Processing factors in development of solid solution formulation of itraconazole for enhancement of drug dissolution and bioavailability. *Int J Pharm.* 2001; 229: 193-203.
25. Zerrouk N, Toscani S, Gines DJ, Chemtob C, Ceólin R, Dugué J. Interactions between carbamazepine and polyethylene glycol (PEG) 6000: Characterizations of the physical, solid dispersed and eutectic

- mixtures. *Eur J Pharm Sci.* 2001; 12: 395-404.
26. Rao GC, Kumar MS, Mathivanan N, Rao ME. Nanosuspensions as the approach in nanoparticulate drug delivery systems. *Pharmazie.* 2004; 59: 5-9.
 27. Liversidge GG, Cundy KC, Particle size reduction for improvement of oral bioavailability of hydrophobic drugs: I. Absolute oral bioavailability of nanocrystalline danazol in beagle dogs. *Int J Pharm.* 1995; 125: 91-97.
 28. Jinno J, Kamada N, Miyake M, Yamada K, Mukai T, Odomi M, Toguchi H, Liversidge GG, Higaki K, Kimura T. Effect of particle size reduction on dissolution and oral absorption of a poorly water-soluble drug, cilostazol, in beagle dogs. *J Control Release* 2006; 111: 56-64.
 29. Langguth P, Hanafy A, Frenzel D, Grenier P, Nhamias A, Ohlig T, Vergnault G, Spahn LH. Nanosuspension formulations for low-soluble drugs: pharmacokinetic evaluation using spironolactone as model compound. *Drug Dev Ind Pharm.* 2005; 31: 319-329.
 30. Abdul IS, Kittelson DB, Brear F. The influence of dilution conditions on diesel exhaust particle size distribution measurements. *SAE Technical Paper Series.* 1999; 1, 1127-1142.
 31. Mathis U, Mohr M, Zenobi R. Effect of organic compounds on nanoparticle formation in diluted diesel exhaust. *Atmos Chem Phys.* 2004; 4: 609-620
 32. Mavrocordatos D, Kaegi R, Schmatloch V. 2002. Fractal analysis of wood combustion aggregates by contact mode atomic force microscopy. *Atmos Environ.* 2002; 36: 5653–5660.
 33. Gareth AH. Nanostructure-mediated drug delivery Nanomedicine: Nanotechnology, Biology and Medicine. 2005; 1: 22-30.
 34. Müller RH, Radtke M, Wissing SA. Nanostructured lipid matrices for improved microencapsulation of drugs. *Int J Pharm.* 2002; 242: 121-128.
 35. Kathy WY, Tri HN, Tracey H, Ben JB. Nanostructure of liquid crystalline matrix determines in vitro sustained release and in vivo oral absorption kinetics for hydrophilic model drugs. *Int J Pharm.* 2009; 365: 190-199.
 36. Shram, M, Romach M, Sellers E, Thippawong J. Assessing the abuse potential of an oral osmotic-controlled extended release (OROS) hydromorphone compared to immediate release hydromorphone. *The Journal of Pain.* 2009; 10: 45-54.
 37. Zentner GM, Rork GS, Himmelstein KJ. The controlled porosity osmotic pump. *J Control Rel.* 1985; 1: 269-282.
 38. Carrigan PJ, Bates TR. Biopharmaceutics of drug administered in lipid- containing dosage forms, part I: GI absorption of griseofulvin from an oil-in-water emulsion in the rat. *J Pharm Sci.* 1973; 62: 1477-1481.
 39. Noguchi T, Takahashi C, Kimura T, Muranishi S, Sezaki H. Mechanism of the intestinal absorption of drugs from oil-in-water emulsions. *Chem Pharm Bull.* 1975; 23: 775-781.
 40. Constantinides PP. Lipid microemulsions for improving drug dissolution and oral absorption: physical and biopharmaceutical aspects. *Pharm Res.* 1995; 12: 156-162.
 41. Colin WP. Formulation of self-emulsifying drug delivery systems. *Adv Drug Deliv Rev.* 1997; 25: 47-58.
 42. Neslihan GR, Simon B. Self-emulsifying drug delivery systems (SEDDS) for improved oral delivery of lipophilic drugs. *Biomedicine & Pharmacotherapy.* 2004; 58: 173-178.
 43. Colin WP. Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *Eur J Pharm Sci.* 2000; 11: 93-98.

44. Bo T, Gang C, Jian CG, Cai HX. Development of solid self-emulsifying drug delivery systems: preparation techniques and dosage forms. *Drug Discov Today*. 2008; 13: 606-612.
45. Shah NH, Carvajal MT, Patel CI, Infeld MH, Malick AW. Self-emulsifying drug delivery systems (SEDDS) with polyglycolized glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. *Int J Pharm*. 1994; 106: 15-23.
46. Anthony A, Attama N, Megg ON. In vitro evaluation of drug release from self micro-emulsifying drug delivery systems using a biodegradable homolipid from *Capra hircus*. *Int J Pharm*. 2005; 304: 4-10.

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ANNEX B

GOODMAN & GILMAN'S The PHARMACOLOGICAL BASIS OF THERAPEUTICS

Ninth Edition

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Lens. The lens, a transparent biconvex structure, is suspended by zonules, specialized collagenous fibers emanating from the ciliary body. The lens is approximately 10 mm in diameter and is enclosed in a capsule. The bulk of the lens is composed of fibers derived from proliferating lens epithelial cells located under the anterior portion of the lens capsule. These lens fibers are continuously produced throughout life.

Posterior Segment. Because of the anatomical and vascular barriers to both local and systemic access, drug delivery to the eye's posterior pole is particularly challenging.

Sclera. The outermost coat of the eye, the sclera, covers the posterior portion of the globe. The external surface of the scleral shell is covered by an episcleral vascular coat, by Tenon's capsule, and by the conjunctiva. The tendons of the six extraocular muscles insert into the superficial scleral collagen fibers. Numerous blood vessels pierce the sclera through emissaria to supply as well as drain the choroid, ciliary body, optic nerve, and iris.

Inside the scleral shell, the vascular choroid nourishes the outer retina by a capillary system in the choriocapillaris. Between the outer retina and the choriocapillaris lies Bruch's membrane and the retinal pigment epithelium, whose tight junctions provide an outer barrier between the retina and the choroid. The retinal pigment epithelium serves many functions, including vitamin A metabolism (see Chapter 63), phagocytosis of the rod outer segments, and multiple transport processes.

Retina. The retina is a thin, transparent, highly organized structure of neurons, glial cells, and blood vessels. Of all structures within the eye, the neurosensory retina has been the most widely studied (see Dowling, 1987). The unique organization and biochemistry of the photoreceptors have provided a superb model for investigating signal transduction mechanisms (see Stryer, 1987). Rhodopsin has been intensely analyzed at the level of its protein and gene structures (see Khorana, 1992). The wealth of information about rhodopsin has made it an excellent model for the G protein-coupled receptors (see Chapter 2). Such detailed understanding holds promise for targeted therapy for some of the hereditary retinal diseases.

Vitreous. The vitreous is a clear medium that makes up about 80% of the eye's volume. It is composed of 99% water bound with collagen type II, hyaluronic acid, and proteoglycans. The vitreous also contains glucose, ascorbic acid, amino acids, and a number of inorganic salts (see Sebag, 1989). Very little is known about the molecular biochemistry or synthesis of vitreous.

Optic Nerve. The optic nerve is a myelinated nerve conducting the retinal output to the central nervous system. It is composed of (1) an intraocular portion, which is visible as the 1.5-mm optic disc in the retina, (2) an intraorbital portion, (3) an intracanalicular portion, and (4) an intracranial portion. The nerve is ensheathed in meninges continuous with the brain. At present, pharmacological treatment of some optic neuropathies is based on management of underlying disease. For example, optic neuritis may be treated by intravenous steroids (Beck *et al.*, 1992); glaucomatous optic neuropathy is medically managed by decreasing intraocular pressure.

PHARMACOKINETICS AND TOXICOLOGY OF OCULAR THERAPEUTIC AGENTS

Drug Delivery Strategies

Factors that affect the bioavailability of ocular drugs include pH, salt form of drug, various structural forms of a given drug, vehicle composition, osmolality, tonicity, and viscosity. Properties of varying ocular routes of administration are outlined in Table 65-3. A number of delivery systems have been developed for treating ocular diseases. Most ophthalmic drugs are delivered in aqueous solutions. For compounds with limited solubility, a suspension form facilitates delivery.

Several formulations prolong the time a drug remains on the surface of the eye. These include gels, ointments, solid inserts, soft contact lenses, and collagen shields. Prolonging the time in the cul-de-sac facilitates drug absorption. Ophthalmic gels (e.g., pilocarpine 4% gel) release drugs by diffusion following erosion of soluble polymers. The polymers used include cellulosic ethers, polyvinyl alcohol, carbopol, polyacrylamide, polymethylvinyl ether-maleic anhydride, poloxamer 407, and puronic acid. Ointments usually contain mineral oil and a petrolatum base and are helpful in delivering antibiotics, cycloplegic drugs, or miotic agents when patching the eye. Solid inserts, such as OCUSERT PILO-20 and PILO-40, have the advantage of providing zero-order rate of delivery by steady-state diffusion. In zero-order delivery, a constant rate of drug is released to the precorneal tear film over a finite period of time. For instance, OCUSERT PILO-20 releases pilocarpine at the rate of 20 $\mu\text{g}/\text{hour}$. Such an approach helps to reduce the side effects of a drug like pilocarpine; a single drop of a 2% solution would deliver a 20-mg bolus to the ocular tissues. Although membrane-controlled drug delivery has many advantages, patients often have difficulty placing and retain-

physician should be aware of the different potentially pathogenic flora, *e.g.*, *Haemophilus influenzae*, and the risk of orbital cellulitis. In adults, dacryocystitis and canaliculitis may be caused by *Staphylococcus aureus*, *Streptococcus* species, *Candida* species, and *Actinomyces israelii*.

Infectious processes of the lids include *hordeolum* and *blepharitis*. A hordeolum, or sty, is an infection of the meibomian, Zeis, or Moll glands at the lid margins. The typical offending bacteria is *Staphylococcus aureus*, and the usual treatment consists of warm compresses and topical antibiotic ointment. *Blepharitis* is a common bilateral inflammatory process of the eyelids characterized by irritation and burning, and it also is usually caused by a *Staphylococcus* species. Local hygiene is the mainstay of therapy; topical antibiotics frequently are used, usually in ointment form, particularly when the disease is accompanied by conjunctivitis and *keratitis*.

The most common eye disease worldwide, *conjunctivitis*, is an inflammatory process of the conjunctiva which varies in severity from mild hyperemia to severe purulent discharge. The causes of conjunctivitis include infectious pathogens, immune-mediated reactions, chemicals/drugs, associated systemic diseases, and tumors of the conjunctiva or eyelid. The more commonly reported infectious agents are *Neisseria* species, *Streptococcus pneumoniae*, *Haemophilus* species, *Staphylococcus aureus*, *Moraxella lacunata*, chlamydial species, adenovirus, herpes simplex virus, enterovirus, coxsackievirus, measles virus, varicella-zoster virus, and vaccinia-variola virus. *Rickettsia*, fungi, and parasites, in both cyst and trophozoite form, are rare causes of conjunctivitis. Effective therapy is based on selection of appropriate antibiotic therapy against a given bacterial pathogen. However, unless an unusual causative organism is suspected, bacterial conjunctivitis is initially treated empirically, without obtaining a culture.

Keratitis, or corneal ulcer, may occur at any level of the cornea, *e.g.*, epithelium, subepithelium, stroma, or endothelium. Numerous microbial agents have been isolated, including bacteria, viruses, fungi, spirochetes, and cysts and trophozoites. In aggressive forms of bacterial keratitis, immediate empirical and intensive antibiotic/antiviral therapy is essential to prevent blindness from corneal perforation; results of culture and sensitivity tests should guide the final drug of choice.

Endophthalmitis is a potentially severe and devastating inflammatory, and usually infectious, process of the posterior segment of the eye. It usually is caused by bacteria, by fungi, or sometimes by spirochetes. The typical case occurs during the early postoperative course (*e.g.*, af-

ter cataract, glaucoma, cornea, or retinal surgery), following trauma, or by endogenous seeding in the immunocompromised host. Prompt therapy usually includes vitrectomy (*i.e.*, specialized surgical removal of the vitreous) and empirical intravitreal antibiotics to treat suspected bacterial or fungal microorganisms (*see* Peyman and Schulman, 1994; Meredith, 1994). In cases of endogenous seeding, parenteral antibiotics most certainly have a role in eliminating the infectious source. In trauma or in the postoperative setting, however, the efficacy of systemic antibiotics is not well established. A multicenter study recently has been carried out to determine whether early intervention with a vitrectomy versus a vitreous tap, both with intravitreal antibiotics and with or without intravenous antibiotic supplements, affects outcome (Endophthalmitis Vitrectomy Study, 1993).

Antiviral Agents. General Considerations. The AIDS epidemic and the emergence of other immunocompromised hosts (*e.g.*, patients who have had organ transplants or those undergoing chemotherapy for malignancies) have spurred research in virology. The challenge of antiviral drug development relates to understanding and targeting molecular events involved in viral adherence, uncoating, replication in host cells, and specific cell-virus biology. The various antiviral drugs currently used in ophthalmology are summarized in Table 65-6 (*see* Chapter 50 for structural formulas and additional details about these agents).

Therapeutic Uses. The primary indications for the use of antiviral drugs in ophthalmology are viral keratitis, herpes zoster ophthalmicus, and retinitis (*see* Teich *et al.*, 1992; Paván-Langston, 1994; Pinnolis *et al.*, 1994; Blumenkranz *et al.*, 1994). Viral conjunctivitis caused by adenoviruses usually has a self-limited course and typically is treated by symptomatic relief of irritation.

Viral keratitis, an infection of the cornea that may involve either the epithelium or stroma, is most commonly caused by herpes simplex type 1 and varicella-zoster viruses. Less common viral etiologies include Epstein-Barr virus and cytomegalovirus. When treating viral keratitis topically, there is a very narrow margin between the therapeutic topical antiviral activity and the toxic effect on the cornea; hence, patients must be followed very closely.

Herpes-zoster-ophthalmicus is a latent reactivation of a varicella-zoster infection in the first division of the trigeminal cranial nerve. Systemic acyclovir is effective in reducing the severity and complications of herpes zoster ophthalmicus (Cobo *et al.*, 1986). Currently, there are no FDA-approved ophthalmic preparations of acyclovir, al-

Use of Autonomic Agents in the Eye

General Considerations. General autonomic pharmacology has been discussed extensively in Chapters 6 through 10. The autonomic agents used in ophthalmology as well as the responses (*i.e.*, mydriasis and cycloplegia) to muscarinic cholinergic antagonists are summarized in Table 65-8.

Therapeutic Uses. Autonomic drugs are used extensively for diagnostic and surgical purposes and for the treatment of glaucoma, uveitis, and strabismus.

Glaucoma. In the United States, glaucoma is the leading cause of blindness in African-Americans and the third leading cause in Caucasians. Characterized by progressive optic nerve cupping and visual field loss, glaucoma is responsible for visual impairment of 80,000 Americans, and at least two million have the disease (*see* American Academy of Ophthalmology, 1992). Risk factors associated with glaucomatous nerve damage include increased intraocular pressure, positive family history of glaucoma, African-American heritage, myopia, and hypertension. The production and regulation of aqueous humor have been discussed in an earlier section of this chapter. Although particularly elevated intraocular pressures (*e.g.*, greater than 30 mm Hg) usually will lead to optic nerve damage, certain patients' optic nerves appear to be able to tolerate intraocular pressures in the mid-to-high twenties. These patients are referred to as *ocular hypertensives*, and a prospective multicenter study is currently under way to determine whether or not early medical treatment to lower intraocular pressure will prevent glaucomatous optic nerve damage. Other patients have progressive glaucomatous optic nerve damage despite having intraocular pressures in the so-called normal range, and this form of the disease is sometimes called *normal tension* or *low tension* glaucoma. However, at present, the pathophysiological processes involved in glaucomatous optic nerve damage and the relationship to aqueous humor dynamics are not understood.

Current medical therapies are targeted to decrease the production of aqueous humor at the ciliary body and to increase outflow of this fluid from the angle-structures. Acute intraocular pressure elevation caused by angle-closure glaucoma is managed with topical β -adrenergic antagonists, low concentrations of pilocarpine (*i.e.*, not higher than 2%, since higher concentrations can cause increased pupillary block and aggravate the acute glaucoma), apraclonidine, acetazolamide either intravenously or orally, and may possibly require the use of an oral or intravenous osmotic agent. The sudden and marked intraocular pressure elevation that occurs in acute angle-closure glaucoma can disrupt the corneal endothelial pump function, leading to corneal edema. Part of the goal of pharmacological inter-

vention is to clear the cornea to help in delivering definitive therapy for this disorder. Once the attack is "broken," a curative laser iridotomy may be performed.

As a caveat mentioned in other chapters and implied on a number of drug warning labels, acute angle-closure glaucoma may be induced in anatomically predisposed eyes by anticholinergic, sympathomimetic, and antihistaminic agents. However, on these warning labels, the type of glaucoma is not specified, and undue anxiety is provoked among patients who have the open-angle form of glaucoma and who need not be concerned about taking these classes of drugs. However, in susceptible eyes, these drugs can lead to partial dilation of the pupil and a change in the vectors of force between the iris and the lens. The aqueous humor then is prevented from passing through the pupil from the posterior chamber to the anterior chamber. The result can be an increase in pressure in the posterior chamber, causing the iris base to be pushed against the angle wall, thereby closing the filtration angle and markedly elevating intraocular pressure.

For medical management of open-angle glaucoma, the selection process for a particular drug class and combination therapy depends on the patient's health, age, and ocular status. Some general principles prevail in patient management: (1) young patients usually are intolerant of miotic therapy secondary to visual blurring from induced myopia; therefore, the OCUSERT delivery system usually is preferable in younger patients; (2) asthma and chronic obstructive pulmonary emphysema having a bronchospastic component are relative contraindications to the use of topical β -adrenergic antagonists because of the risk of significant side effects from systemic absorption *via* the nasolacrimal system; (3) cardiac dysrhythmias (*i.e.*, bradycardia and heart block) and congestive heart failure also are relative contraindications for similar reasons; (4) history of nephrolithiasis, or kidney stones, is sometimes a contraindication for carbonic anhydrase inhibitors; (5) direct miotic agents are preferred over cholinesterase inhibitors in "phakic" patients (*i.e.*, those patients who have their endogenous lens), since the latter drugs are associated with cataract formation; and (6) in patients who have an increased risk of retinal detachment, miotics should be used with caution, since they have been implicated in promoting retinal tears in susceptible individuals; such tears are thought to be due to altered forces at the vitreous base produced by ciliary body contraction induced by the drug.

There is no consensus on medical therapy for glaucoma. The ophthalmologist must consider compliance issues and the aforementioned medical concerns, along with the quality-of-life alterations caused by the disease and its treatment when recommending a particular therapy to a patient. Typically, a stepped medical approach is taken with the main goal of preventing progressive glaucomatous optic nerve damage with minimum risk and side effects from either topical or systemic therapy. Usually, topical therapy is initiated with a β -adrenergic antagonist. If the targeted pressure-reduction goals are not achieved, a cholinergic agonist (*e.g.*, pilocarpine or carbachol) or a cholinesterase inhibitor may be added depending on the status of the patient's lens. Ironically, epinephrine-related drugs may be used concomitantly with a β -adrenergic antagonist. Epinephrine's effect may be mediated by reducing blood flow in the ciliary body and consequently suppressing aqueous humor production. The introduction in 1993 of *apraclonidine* (IOPIDINE), an α_2 -adrenergic agonist, provides another pharmacological approach to reduce aqueous humor production. If combined topical therapy fails to achieve the target intraocular pressure or fails to halt glaucomatous optic nerve damage, then systemic therapy with carbonic anhydrase inhibitors may be initiated. Of the oral preparations available (*e.g.*, methazolamide, acetazolamide, and dichlorphenamide), the best tolerated is acetazol-

ANNEX C

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Normal-Tension Glaucoma



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Normal-Tension Glaucoma Overview

Glaucoma is usually high pressure inside the eye that damages the optic nerve and can result in permanent vision loss. Normal-tension glaucoma (also called low-tension glaucoma) is a unique condition in which optic nerve damage and vision loss have occurred despite a normal pressure inside the eye.

Eye pressure, called intraocular pressure (IOP), is measured in millimeters of mercury (mm Hg). Normal eye pressure ranges from 10-21 mm Hg. Most people with glaucoma have IOP of greater than 21 mm Hg; however, in normal-tension glaucoma, people have IOP within the normal range.

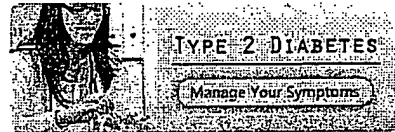
By definition, people with normal-tension glaucoma have open, normal-appearing angles. In fact, the features of normal-tension glaucoma are similar to primary open-angle glaucoma (POAG), the most common form of glaucoma (see Primary Open-Angle Glaucoma).

- Although the occurrence of normal-tension glaucoma varies worldwide, it is very prevalent in Japan.
- In the United States, up to 15-25% of people with open-angle glaucoma experience normal-tension glaucoma.
- According to the Baltimore Eye Study, 50% of individuals with changes in their optic disc (the front surface of the optic nerve) and in their visual field had an IOP of less than 21 mm Hg on a single visit, and 33% had an IOP of less than 21 mm Hg on 2 measurements.
- Normal-tension glaucoma is more common in women than in men.
- Normal-tension glaucoma affects adults, with an average age of 60 years.

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Normal-Tension Glaucoma

Adult Glaucoma Suspect »

Adult Glaucoma Suspect Overview

Glaucoma is usually high pressure inside the eye that damages the optic nerve and can result in permanent vision loss. Not all 3 criteria (that is, high pressure inside the eye, optic nerve damage, and vision loss) are required to diagnose glaucoma; however, a diagnosis of glaucoma is certain when all 3 criteria are present.

Elevated pressure inside the eye, called intraocular pressure (IOP), is a primary concern because it is one of the main risk factors for glaucoma. In fact, the prevalence of primary open-angle glaucoma (POAG), the most common form of glaucoma, is higher with increasing IOP.

Eye pressure is measured in millimeters of mercury (mm Hg). Normal eye pressure ranges from 10-21 mm Hg. Elevated IOP is a pressure of greater than 21 mm Hg. The term ocular hypertension (OHT) refers to any situation in which IOP is higher than normal.

Glaucoma suspect describes a person with one or mor...

ANNEX D

Glaucoma

From Wikipedia, the free encyclopedia

Glaucoma is an eye disorder in which the optic nerve suffers damage, permanently impacting vision in the affected eye(s) and progressing to complete blindness if untreated. It is often, but not always, associated with increased pressure of the fluid in the eye (aqueous humour).^[1]

The nerve damage involves loss of retinal ganglion cells in a characteristic pattern. There are many different sub-types of glaucoma but they can all be considered a type of optic neuropathy. Raised intraocular pressure is a significant risk factor for developing glaucoma (above 21 mmHg or 2.8 kPa). One person may develop nerve damage at a relatively low pressure, while another person may have high eye pressure for years and yet never develop damage. Untreated glaucoma leads to permanent damage of the optic nerve and resultant visual field loss, which can progress to blindness.

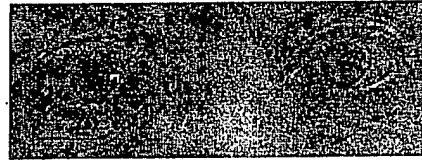
Glaucoma can be divided roughly into two main categories, "open angle" and "closed angle" glaucoma. Closed angle glaucoma can appear suddenly and is often painful; visual loss can progress quickly but the discomfort often leads patients to seek medical attention before permanent damage occurs. Open angle, chronic glaucoma tends to progress at a slower rate and the patient may not notice that they have lost vision until the disease has progressed significantly.

Glaucoma has been nicknamed the "silent thief of sight" because the loss of vision normally occurs gradually over a long period of time and is often only recognized when the disease is quite advanced. Once lost, this damaged visual field cannot be recovered. Worldwide, it is the second leading cause of blindness.^[2] It is also the first leading cause of blindness among African Americans.^[3] Glaucoma affects 1 in 200 people aged fifty and younger, and 1 in 10 over the age of eighty. If the condition is detected early enough it is possible to arrest the development or slow the progression with medical and surgical means.

The word *glaucoma* comes from the Greek γλαύκωμα, "opacity of the crystalline lens".^[4]

Glaucoma

Classification and external resources



Acute angle closure glaucoma of the right eye. Note the mid sized pupil, which was non-reactive to light, and infection of the conjunctiva.

ICD-10	H40. (http://apps.who.int/classifications/apps/icd/icd10online/?gh40.htm+h40) -H42. (http://apps.who.int/classifications/apps/icd/icd10online/?gh40.htm+h42)
ICD-9	365 (http://www.icd9data.com/getICD9Code.aspx?icd9=365)
DiseasesDB	5226 (http://www.diseasesdatabase.com/ddb5226.htm)
eMedicine	oph/578 (http://www.emedicine.com/oph/topic578.htm)
MeSH	D005901 (http://www.nlm.nih.gov/cgi/mesh/2010/MB_cgi?field=uid&term=D005901)

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Signs and symptoms

There are two main types of glaucoma: **open-angle glaucoma** and **closed-angle glaucoma**.

Open-angle glaucoma accounts for 90% of glaucoma cases in the United States. It is painless and does not have acute attacks. The only signs are gradually progressive visual field loss, and optic nerve changes (increased cup-to-disc ratio on fundoscopic examination).

Closed-angle glaucoma accounts for less than 10% of glaucoma cases in the United States, but as much as half of glaucoma cases in other nations (particularly Asian countries). About 10% of patients with closed angles present with acute angle closure crises characterized by sudden ocular pain, seeing halos around lights, red eye, very high intraocular pressure (>30 mmHg), nausea and vomiting, sudden decreased vision, and a fixed, mid-dilated pupil. Acute angle closure is an ocular emergency.

Pathophysiology

The major risk factor for most glaucomas and focus of treatment is increased intraocular pressure. Intraocular pressure is a function of production of liquid aqueous humor by the ciliary processes of the eye and its drainage through the trabecular meshwork. Aqueous humor flows from the ciliary processes into the posterior chamber, bounded posteriorly by the lens and the zonules of Zinn and anteriorly by the iris. It then flows through the pupil of the iris into the anterior chamber, bounded

posteriorly by the iris and anteriorly by the cornea. From here the trabecular meshwork drains aqueous humor via Schlemm's canal into scleral plexuses and general blood circulation.^[5] In open angle glaucoma there is reduced flow through the trabecular meshwork;^[6] in angle closure glaucoma, the iris is apposed to the lens resulting in the inability of the aqueous fluid to flow from the posterior to the anterior chamber and then out of the trebecular network.

The inconsistent relationship of glaucomatous optic neuropathy with ocular hypertension has provoked hypotheses and studies on anatomic structure, eye development, nerve compression trauma, optic nerve blood flow, excitatory neurotransmitter, trophic factor, retinal ganglion cell/axon degeneration, glial support cell, immune, and aging mechanisms of neuron loss.^{[7][8][9][10][11][12][13][14][15][16][17]}

The major types of glaucoma are discussed below.

Causes and risk factors

There are several causes for glaucoma. *Those at risk are advised to have a dilated eye examination at least once a year.*^[18]

Ocular hypertension (increased pressure within the eye) is the largest risk factor in most glaucomas, but in some populations only 50% of patients with primary open angle glaucoma actually have elevated ocular pressure.^[19]

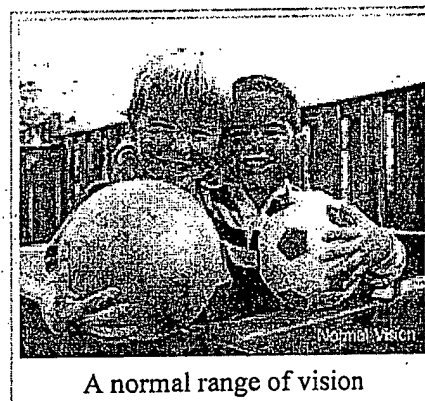
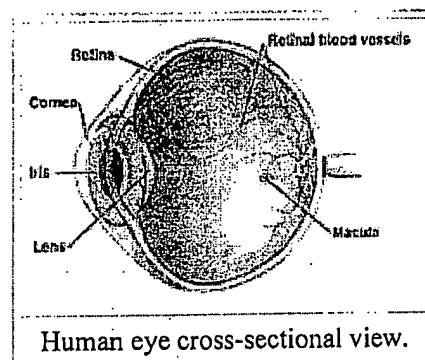
Those of African descent are three times more likely to develop primary open angle glaucoma.

Elder people have thinner corneal thickness and often suffer from hypermetropia. They are also at higher risk for primary open angle glaucoma.

People with a family history of glaucoma have about six percent chance of developing glaucoma.

Many East Asian groups are prone to developing angle closure glaucoma due to their shallower anterior chamber depth, with the majority of cases of glaucoma in this population consisting of some form of angle closure.^[20] Inuit also have a twenty to forty times higher risk than Caucasians of developing primary angle closure glaucoma. Women are three times more likely than men to develop acute angle-closure glaucoma due to their shallower anterior chambers.

Other factors can cause glaucoma, known as "secondary glaucomas," including prolonged use of steroids (steroid-induced glaucoma); conditions that severely restrict blood flow to the eye, such as severe diabetic retinopathy and central retinal vein occlusion (neovascular glaucoma); ocular trauma (angle recession glaucoma); and uveitis (uveitic glaucoma).



Primary open angle glaucoma (POAG) has been found to be associated with mutations in genes at several loci.^[21] Normal tension glaucoma, which comprises one third of POAG, is associated with genetic mutations.^[22]

There is increasing evidence that ocular blood flow is involved in the pathogenesis of glaucoma. Current data indicate that fluctuations in blood flow are more harmful in glaucomatous optic neuropathy than steady reductions. Unstable blood pressure and dips are linked to optic nerve head damage and correlate with visual field deterioration.

A number of studies also suggest a possible correlation between hypertension and the development of glaucoma. In normal tension glaucoma, nocturnal hypotension may play a significant role.

There is no clear evidence that vitamin deficiencies cause glaucoma in humans. It follows then that oral vitamin supplementation is not a recommended treatment for glaucoma.^[23]

Various rare congenital/genetic eye malformations are associated with glaucoma. Occasionally, failure of the normal third trimester gestational atrophy of the hyaloid canal and the tunica vasculosa lentis is associated with other anomalies. Angle closure induced ocular hypertension and glaucomatous optic neuropathy may also occur with these anomalies.^{[24][25][26]} and modelled in mice.^[27]

Diagnosis

Screening for glaucoma is usually performed as part of a standard eye examination performed by ophthalmologists, orthoptists and optometrists. Testing for glaucoma should include measurements of the intraocular pressure via tonometry, changes in size or shape of the eye, anterior chamber angle examination or gonioscopy, and examination of the optic nerve to look for any visible damage to it, or change in the cup-to-disc ratio and also rim appearance and vascular change. A formal visual field test should be performed. The retinal nerve fiber layer can be assessed with imaging techniques such as optical coherence tomography (OCT), scanning laser polarimetry (GDx), and/or scanning laser ophthalmoscopy also known as Heidelberg Retina Tomography (HRT3).^{[28][29]} Owing to the sensitivity of all methods of tonometry to corneal thickness, methods such as Goldmann tonometry should be augmented with pachymetry to measure central corneal thickness (CCT). A thicker-than-average cornea can result in a pressure reading higher than the 'true' pressure, whereas a thinner-than-average cornea can produce a pressure reading lower than the 'true' pressure. Because pressure measurement error can be caused by more than just CCT (i.e., corneal hydration, elastic properties, etc.), it is impossible to 'adjust' pressure measurements based only on CCT measurements. The Frequency Doubling Illusion can also be used to detect glaucoma with the use of a Frequency Doubling Technology (FDT) perimeter.^[30] Examination for glaucoma also could be assessed with more attention given to sex, race, history of drug use, refraction, inheritance and family history.^[28]

Management

The modern goals of glaucoma management are to avoid glaucomatous damage, nerve damage, preserve visual field and total quality of life for patients with minimal side effects.^{[31][32]} This requires appropriate diagnostic techniques and follow up examinations and judicious selection of treatments for the individual patient. Although intraocular pressure is only one of the major risk factors for glaucoma, lowering it via various pharmaceuticals and/or surgical techniques is currently the mainstay of glaucoma treatment. Vascular flow and neurodegenerative theories of glaucomatous optic neuropathy have prompted studies on various neuroprotective therapeutic strategies including nutritional compounds some of which may be regarded by clinicians as safe for use now, while others are on trial.

Medication

Intraocular pressure can be lowered with medication, usually eye drops. There are several different classes of medications to treat glaucoma with several different medications in each class.

Each of these medicines may have local and systemic side effects. Adherence to medication protocol can be confusing and expensive; if side effects occur, the patient must be willing either to tolerate these, or to communicate with the treating physician to improve the drug regimen. Initially, glaucoma drops may reasonably be started in either one or in both eyes.^[33]

Poor compliance with medications and follow-up visits is a major reason for vision loss in glaucoma patients. A 2003 study of patients in an HMO found that half failed to fill their prescription the first time and one in four failed to refill their prescriptions a second time.^[34] Patient education and communication must be ongoing to sustain successful treatment plans for this lifelong disease with no early symptoms.

The possible neuroprotective effects of various topical and systemic medications are also being investigated.^{[23][35][36][37]}

- Prostaglandin analogs like latanoprost (Xalatan), bimatoprost (Lumigan) and travoprost (Travatan) increase uveoscleral outflow of aqueous humor. Bimatoprost also increases trabecular outflow
- Topical beta-adrenergic receptor antagonists such as timolol, levobunolol (Betagan), and betaxolol decrease aqueous humor production by the ciliary body.
- Alpha2-adrenergic agonists such as brimonidine (Alphagan) work by a dual mechanism, decreasing aqueous humor production and increasing trabecular outflow.
- Less-selective sympathomimetics such as epinephrine decrease aqueous humor production through vasoconstriction of ciliary body blood vessels.
- Miotic agents (parasympathomimetics) like pilocarpine work by contraction of the ciliary muscle, tightening the trabecular meshwork and allowing increased outflow of the aqueous humour. Ecothiopate is used in chronic glaucoma.
- Carbonic anhydrase inhibitors like dorzolamide (Trusopt), brinzolamide (Azopt), acetazolamide (Diamox) lower secretion of aqueous humor by inhibiting carbonic anhydrase in the ciliary body.
- Physostigmine is also used to treat glaucoma and delayed gastric emptying.

For cannabis as a treatment, see Compounds in Research

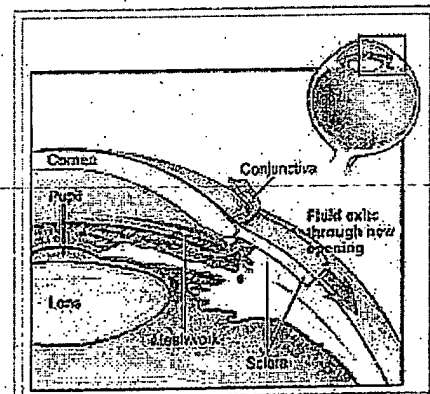
Surgery

Main article: Glaucoma surgery

Both laser surgeries and conventional surgeries are performed to treat glaucoma.

Surgery is the primary therapy for those with congenital glaucoma.^[38]

Generally, these operations are a temporary solution, as there is not yet a cure for glaucoma.



Canaloplasty

Canaloplasty is a nonpenetrating procedure utilizing microcatheter technology. To perform a canaloplasty, an incision is made into the eye to gain access to Schlemm's canal in a similar fashion to a viscocanalostomy. A

microcatheter will circumnavigate the canal around the iris, enlarging the main drainage channel and its smaller collector channels through the injection of a sterile, gel-like material called viscoelastic. The catheter is then removed and a suture is placed within the canal and tightened. By opening the canal, the pressure inside the eye may be relieved, although the reason is unclear since the canal (of Schlemm) does not have any significant fluid resistance in glaucoma or healthy eyes. Long-term results are not available.^{[39][40]}

Conventional surgery to treat glaucoma makes a new opening in the meshwork. This new opening helps fluid to leave the eye and lowers intraocular pressure.

Laser surgery

Laser trabeculoplasty may be used to treat open angle glaucoma. It is a temporary solution, not a cure. A 50 µm argon laser spot is aimed at the trabecular meshwork to stimulate opening of the mesh to allow more outflow of aqueous fluid. Usually, half of the angle is treated at a time. Traditional laser trabeculoplasty utilizes a thermal argon laser. The procedure is called Argon Laser Trabeculoplasty or ALT. A newer type of laser trabeculoplasty exists that uses a "cold" (non-thermal) laser to stimulate drainage in the trabecular meshwork. This newer procedure which uses a 532 nm frequency-doubled, Q-switched Nd:YAG laser which selectively targets melanin pigment in the trabecular meshwork cells, called Selective Laser Trabeculoplasty or SLT. Studies show that SLT is as effective as ALT at lowering eye pressure. In addition, SLT may be repeated three to four times, whereas ALT can usually be repeated only once.

Nd:YAG laser peripheral iridotomy (LPI) may be used in patients susceptible to or affected by angle closure glaucoma or pigment dispersion syndrome. During laser iridotomy, laser energy is used to make a small full-thickness opening in the iris. This opening equalizes the pressure between the front and back of the iris correcting any abnormal bulging of the iris. In people with narrow angles, this can uncover the trabecular meshwork. In some cases of intermittent or short-term angle closure this may lower the eye pressure. Laser iridotomy reduces the risk of developing an attack of acute angle closure. In most cases it also reduces the risk of developing chronic angle closure or of adhesions of the iris to the trabecular meshwork.

Diode laser cycloablation lowers IOP by reducing aqueous secretion by destroying secretory ciliary epithelium.^[28]

Trabeculectomy

The most common conventional surgery performed for glaucoma is the trabeculectomy. Here, a partial thickness flap is made in the scleral wall of the eye, and a window opening made under the flap to remove a portion of the trabecular meshwork. The scleral flap is then sutured loosely back in place. This allows fluid to flow out of the eye through this opening, resulting in lowered intraocular pressure and the formation of a bleb or fluid bubble on the surface of the eye. Scarring can occur around or over the flap opening, causing it to become less effective or lose effectiveness altogether.

Glaucoma drainage implants

There are also several different glaucoma drainage implants. These include the original Molteno implant (1966), the Baerveldt tube shunt, or the valved implants, such as the Ahmed glaucoma valve implant or the ExPress Mini Shunt and the later generation pressure ridge Molteno implants. These are indicated for glaucoma patients not responding to maximal medical therapy, with previous failed

guarded filtering surgery (trabeculectomy). The flow tube is inserted into the anterior chamber of the eye and the plate is implanted underneath the conjunctiva to allow flow of aqueous fluid out of the eye into a chamber called a bleb.

- The first-generation Molteno and other non-valved implants sometimes require the ligation of the tube until the bleb formed is mildly fibrosed and water-tight^[41] This is done to reduce postoperative hypotony—sudden drops in postoperative intraocular pressure (IOP).
- Valved implants such as the Ahmed glaucoma valve attempt to control postoperative hypotony by using a mechanical valve.

The ongoing scarring over the conjunctival dissipation segment of the shunt may become too thick for the aqueous humor to filter through. This may require preventive measures using anti-fibrotic medication like 5-fluorouracil (5-FU) or mitomycin-C (during the procedure), or additional surgery. And for Glaucomatous painful Blind Eye and some cases of Glaucoma, Cyclocryotherapy for ciliary body ablation could be considered to be performed.^[28]

Veterinary implant

TR BioSurgical has commercialized a new implant specifically for veterinary medicine, called TR-ClarifEYE. The implant consists of a new biomaterial, the STAR BioMaterial, which consists of silicone with a very precise homogenous pore size, a property which reduces fibrosis and improves tissue integration. The implant contains no valves and is placed completely within the eye without sutures. To date, it has demonstrated long term success (> 1yr) in a pilot study in medically refractory dogs with advanced glaucoma^[42]

Laser assisted non-penetrating deep sclerectomy

The most common surgical approach currently used for the treatment of glaucoma, is trabeculectomy, in which the sclera is punctured to alleviate intraocular pressure (IOP), the pressure inside the eye. Non-penetrating deep sclerectomy (NPDS) surgery is a similar but modified procedure, in which instead of puncturing the scleral wall, a patch of the sclera is skimmed to a level, upon which, percolation of liquid from the inner eye is achieved and thus alleviating IOP, without penetrating the eye. NPDS is demonstrated to cause a significantly less side effects than trabeculectomy.^[citation needed] However, NPDS is performed manually and requires great skill to achieve a lengthy learning curve.^[citation needed]

Laser assisted NPDS is the performance of NPDS with the use of a CO₂ laser system. The laser-based system is self-terminating once the required scleral thickness and adequate drainage of the intra ocular fluid have been achieved. This self-regulation effect is achieved as the CO₂ laser essentially stops ablating as soon as it comes in contact with the intra-ocular percolated liquid, which occurs as soon as the laser reaches the optimal residual intact layer thickness.

Epidemiology

Research

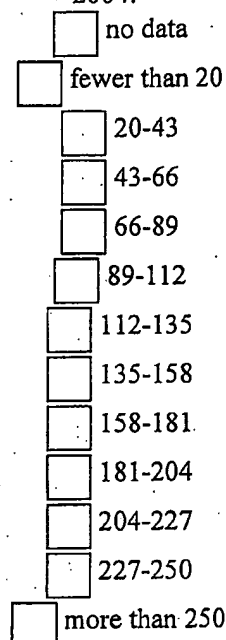
- Advanced Glaucoma Intervention Study (AGIS) (<http://www.nei.nih.gov/neitrials/static/study49.asp>) - large American National Eye Institute (NEI) sponsored study designed "to assess the long-range outcomes of sequences of interventions involving trabeculectomy



and argon laser trabeculoplasty in eyes that have failed initial medical treatment for glaucoma." It recommends different treatments based on race.

- Early Manifest Glaucoma Trial (EMGT) (<http://www.nei.nih.gov/earlyglaucoma/index.asp>) - Another NEI study found that immediately treating people who have early stage glaucoma can delay progression of the disease.
- Ocular Hypertension Treatment Study (OHTS) (<http://www.nei.nih.gov/neitrials/viewStudyWeb.aspx?id=24>) - NEI study findings: "...Topical ocular hypotensive medication was effective in delaying or preventing onset of Primary Open Angle Glaucoma (POAG) in individuals with elevated Intraocular Pressure (IOP). Although this does not imply that all patients with borderline or elevated IOP should receive medication, clinicians should consider initiating treatment for individuals with ocular hypertension who are at moderate or high risk for developing POAG."
- Blue Mountains Eye Study (<http://www.cvr.org.au/bmes.htm>) "The Blue Mountains Eye Study was the first large population-based assessment of visual impairment and common eye diseases of a representative older Australian community sample." Risk factors for glaucoma and other eye disease were determined.

Disability-adjusted life year for glaucoma per 100,000 inhabitants in 2004.^[43]



Compounds in research

Natural compounds

Natural compounds of research interest in glaucoma prevention or treatment include: fish oil and omega 3 fatty acids, bilberries, vitamin E, cannabinoids, carnitine, coenzyme Q10, curcumin, Salvia miltiorrhiza, dark chocolate, erythropoietin, folic acid, Ginkgo biloba, Ginseng, L-glutathione, grape seed extract, green tea, magnesium, melatonin, methylcobalamin, N-acetyl-L cysteine, pycnogenols, resveratrol, quercetin and salt.^{[35][36][37]} Magnesium, ginkgo, salt and fludrocortisone, are already used by some physicians.

Cannabis

Studies in the 1970s showed that marijuana, when smoked, effectively lowers intraocular pressure.^[44] In an effort to determine whether marijuana, or drugs derived from marijuana, might be effective as a glaucoma treatment, the US National Eye Institute supported research studies from 1978 to 1984. These studies demonstrated that some derivatives of marijuana lowered intraocular pressure when administered orally, intravenously, or by smoking, but not when topically applied to the eye.

In 2003 the American Academy of Ophthalmology released a position statement which said that "studies demonstrated that some derivatives of marijuana did result in lowering of IOP when administered orally, intravenously, or by smoking, but not when topically applied to the eye. The duration of the pressure-lowering effect is reported to be in the range of 3 to 4 hours".^{[44][45]}

However, the position paper qualified that by stating that marijuana was not more effective than prescription medications, stating that "no scientific evidence has been found that demonstrates increased benefits and/or diminished risks of marijuana use to treat glaucoma compared with the wide variety of pharmaceutical agents now available."

The first patient in the United States federal government's Compassionate Investigational New Drug program, Robert Randall, was afflicted with glaucoma and had successfully fought charges of marijuana cultivation because it was deemed a medical necessity (*U.S. v. Randall*) in 1976.^[46]

5-HT_{2A} agonists

Peripherally selective 5-HT_{2A} agonists such as the indazole derivative AL-34662 are currently under development and show significant promise in the treatment of glaucoma.^{[47][48]}

Classification

Glaucoma has been classified into specific types:^[49]

Primary glaucoma and its variants (H40.1-H40.2)

- Primary glaucoma
 - Primary angle-closure glaucoma, also known as primary closed-angle glaucoma, narrow-angle glaucoma, pupil-block glaucoma, acute congestive glaucoma
 - Acute angle-closure glaucoma
 - Chronic angle-closure glaucoma
 - Intermittent angle-closure glaucoma
 - Superimposed on chronic open-angle closure glaucoma ("combined mechanism" - uncommon)
 - Primary open-angle glaucoma, also known as chronic open-angle glaucoma, chronic simple glaucoma, glaucoma simplex
 - High-tension glaucoma
 - Low-tension glaucoma
- Variants of primary glaucoma
 - Pigmentary glaucoma
 - Exfoliation glaucoma, also known as pseudoexfoliative glaucoma or glaucoma capsulare

Primary angle-closure glaucoma – This is caused by contact between the iris and trabecular meshwork, which in turn obstructs outflow of the aqueous humor from the eye. This contact between iris and trabecular meshwork (TM) may gradually damage the function of the meshwork until it fails to keep pace with aqueous production, and the pressure rises. In over half of all cases, prolonged contact between iris and TM causes the formation of synechiae (effectively "scars"). These cause permanent obstruction of aqueous outflow. In some cases, pressure may rapidly build up in the eye causing pain and redness (symptomatic, or so called "acute" angle-closure). In this situation the vision may become blurred, and halos may be seen around bright lights. Accompanying symptoms may include headache and vomiting. Diagnosis is made from physical signs and symptoms: pupils mid-dilated and unresponsive to light, cornea edematous (cloudy), reduced vision, redness, pain. However, the majority of cases are asymptomatic. Prior to very severe loss of vision, these cases can only be identified by examination, generally by an eye care professional. Once any symptoms have been controlled, the first line (and often definitive) treatment is laser iridotomy. This may be performed using either Nd:YAG or argon lasers, or in some cases by conventional incisional surgery. The goal of treatment is to reverse, and prevent, contact between iris and trabecular meshwork. In

early to moderately advanced cases, iridotomy is successful in opening the angle in around 75% of cases. In the other 25% laser iridoplasty, medication (pilocarpine) or incisional surgery may be required.

Primary open-angle glaucoma – Optic nerve damage resulting in progressive visual field loss.^[50] This is associated with increased pressure in the eye. Not all people with primary open-angle glaucoma have eye pressure that is elevated beyond normal, but decreasing the eye pressure further has been shown to stop progression even in these cases. The increased pressure is caused by trabecular blockage which is where the aqueous humor in the eye drains out. Because the microscopic passage ways are blocked, the pressure builds up in the eye and causes imperceptible very gradual vision loss. Peripheral vision is affected first but eventually the entire vision will be lost if not treated. Diagnosis is made by looking for cupping of the optic nerve. Prostaglandin agonists work by opening uveoscleral passageways. Beta blockers such as timolol, work by decreasing aqueous formation. Carbonic anhydrase inhibitors decrease bicarbonate formation from ciliary processes in the eye, thus decreasing formation of Aqueous humor. Parasympathetic analogs are drugs that work on the trabecular outflow by opening up the passageway and constricting the pupil. Alpha 2 agonists (brimonidine, apraclonidine) both decrease fluid production (via. inhibition of AC) and increase drainage.

Developmental glaucoma (Q15.0)

- Developmental glaucoma
 - Primary congenital glaucoma
 - Infantile glaucoma
 - Glaucoma associated with hereditary of familial diseases

Secondary glaucoma (H40.3-H40.6)

- Secondary glaucoma
 - Inflammatory glaucoma
 - Uveitis of all types
 - Fuchs heterochromic iridocyclitis
 - Phacogenic glaucoma
 - Angle-closure glaucoma with mature cataract
 - Phacoanaphylactic glaucoma secondary to rupture of lens capsule
 - Phacolytic glaucoma due to phacotoxic meshwork blockage
 - Subluxation of lens
 - Glaucoma secondary to intraocular hemorrhage
 - Hyphema
 - Hemolytic glaucoma, also known as erythroclastic glaucoma
 - Traumatic glaucoma
 - Angle recession glaucoma: Traumatic recession on anterior chamber angle
 - Postsurgical glaucoma
 - Aphakic pupillary block
 - Ciliary block glaucoma

- Neovascular glaucoma (see below for more details)
- Drug-induced glaucoma
 - Corticosteroid induced glaucoma
 - Alpha-chymotrypsin glaucoma. Postoperative ocular hypertension from use of alpha chymotrypsin.
- Glaucoma of miscellaneous origin
 - Associated with intraocular tumors
 - Associated with retinal detachments
 - Secondary to severe chemical burns of the eye
 - Associated with essential iris atrophy
 - Toxic Glaucoma

Neovascular glaucoma is an uncommon type of glaucoma that is difficult or nearly impossible to treat. This condition is often caused by proliferative diabetic retinopathy (PDR) or central retinal vein occlusion (CRVO). It may also be triggered by other conditions that result in ischemia of the retina or ciliary body. Individuals with poor blood flow to the eye are highly at risk for this condition.

Neovascular glaucoma results when new, abnormal vessels begin developing in the angle of the eye that begin blocking the drainage. Patients with such condition begin to rapidly lose their eyesight. Sometimes, the disease appears very rapidly, specially after cataract surgery procedure. A new treatment for this disease, as first reported by Kahook and colleagues, involves use of a novel group of medications known as Anti-VEGF agents. These injectable medications can lead to a dramatic decrease in new vessel formation and, if injected early enough in the disease process, may lead to normalization of intraocular pressure.

Toxic glaucoma is open angle glaucoma with an unexplained significant rise of intraocular pressure following unknown pathogenesis. Intraocular pressure can sometimes reach 80 mmHg (11 kPa). It characteristically manifests as ciliary body inflammation and massive trabecular oedema that sometimes extends to Schlemm's Canal. This condition is differentiated from malignant glaucoma by the presence of a deep and clear anterior chamber and a lack of aqueous misdirection. Also, the corneal appearance is not as hazy. A reduction in visual acuity can occur followed neuroretinal breakdown. Associated factors include inflammation, drugs, trauma and intraocular surgery, including cataract surgery and vitrectomy procedures. Gede Pardianto (2005) reports on four patients who had toxic glaucoma. One of them underwent phacoemulsification with small particle nucleus drops. Some cases can be resolved with some medication, vitrectomy procedures or trabeculectomy. Valving procedures can give some relief but further research is required.^[51]

Absolute glaucoma (H44.5)

- Absolute glaucoma is the end stage of all types of glaucoma. The eye has no vision, absence of PL and PR, and has a stony appearance. Severe pain is present in the eye. The treatment of absolute glaucoma is a destructive procedure like cyclocryo application, cyclophotocoagulation, or injection of 100% alcohol.

ANNEX E

[Print this Page](#)

Presentation Abstract

Program#/Poster#: 2423/A511

Abstract Title: **Solid Lipid Nanoparticles Topically Administered in Rabbits as New Drug Delivery System: A Preliminary Study of Safety and Bioavailability**

Presentation Start/End Time: Monday, May 04, 2009, 3:45 PM - 5:30 PM

Location: Hall B/C

Reviewing Code: 286 nanotechnology (Poster Only) - drug and gene delivery - NT

Author Block: *F. Viola¹, C. Mapelli¹, D. Galimberti¹, G. De Martini^{2A}, R. Esposti^{2B}, L. Moneghini^{2C}, P. Braidotti^{2C}, M. Cresta³, M.R. Gasco⁴, R. Ratiglia¹.* ¹University of Milan, Fondazione IRCCS Ospedale Maggiore Policlinico, Milan, Italy; ^ADipartimento Farmacologia Chemioterapia e Tossicologia Medica, ^BIstituto di Fisiologia Umana II, ^CAnatomia Patologica A.O. S.Paolo, ²University of Milan, Milan, Italy; ³Clinica Vision Vet, Bologna, Italy; ⁴Nanovector srl, Torino, Italy, Italy.

Keywords: 504 drug toxicity/drug effects, 599 microscopy: light/fluorescence/immunohistochemistry, 685 retina

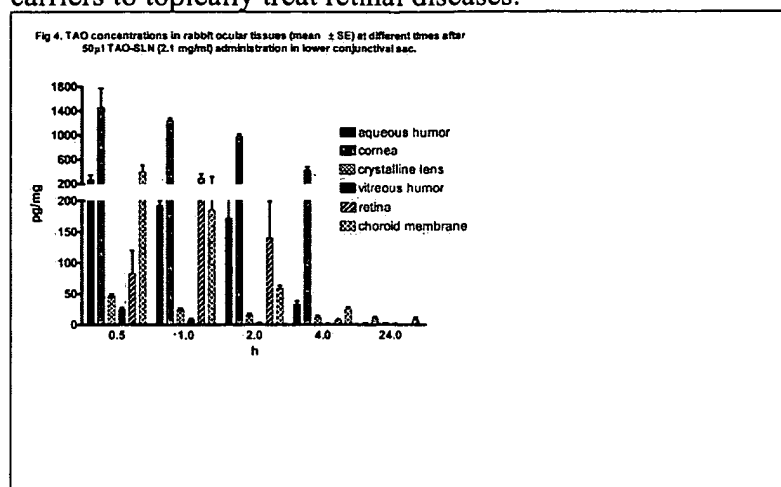
Abstract Body: **Purpose:** Solid lipid nanoparticles (SLN) are new carriers able to incorporate and deliver hydrophobic and hydrophilic drugs. Aim of this work was to evaluate the ocular toxicity and bioavailability of Unloaded-SLN and fluorescent SLN (Fluo-SLN) and to study the bioavailability and pharmacokinetics of Triamcinolone Acetonide-loaded SLN (TA-SLN) in rabbits.

Methods: The different types of SLN were prepared by a warm o/w microemulsion method. Ocular toxicity (assessed by clinical examination, electroretinography, light and electron microscopy) and fluorescence distribution were evaluated 30 minutes, 2, 4 and 24 hours after topical administration of 50 microliter of Unloaded- and Fluo-SLN in 24 eyes. 50 microliter of TA-SLN (2,1 mg/ml) were topically administered in 15 eyes and drug levels in the cornea, aqueous humor, lens, vitreous, retina and choroid were determined at 30 minutes, 1, 2, 4 and 24 hours.

Results: SLN dispersion was perfectly tolerated: there was no evidence of toxic effects based on the clinical examinations. Electroretinography evaluations showed no functional alterations. SLN caused no histopathological or ultrastructural damage to the retina or other ocular tissues. Fluorescence analysis revealed a dotted positivity on cornea surface and a diffuse positivity in retina tissues. Topical administration of TA-SLN resulted in detectable drug levels in the retina and in all the analyzed tissues. Mean concentration \pm SEM in different tissues at different times are reported in Figure 1.

Conclusions: Topically administered SLN are safe and sustain retinal drug delivery. These results add further support to the potential use of these nanoparticles as drug

carriers to topically treat retinal diseases.



Commercial
Relationships:

F. Viola, None; **C. Mapelli**, None; **D. Galimberti**, None; **G. De Martini**, None; **R. Esposti**, None; **L. Moneghini**, None; **P. Braidotti**, None; **M. Cresta**, None; **M.R. Gasco**, Solid lipid nanoparticles by Nanovector, P; **R. Ratiglia**, None.

Support:

RICERCA FINALIZZATA

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ANNEX F

PubMed

U.S. National Library of Medicine
National Institutes of Health

Display Settings: Abstract

Ophthalmologica. 1958 May-Jun;135(5-6):545-54.

[Viral retinitis pigmentosa; relation between clinical picture and electroretinogram].

[Article in French]

FRANCESCHETTI A, DIETERLE P, SCHWARZ A.

PMID: 13553262 [PubMed - OLDMEDLINE]

MeSH Terms

LinkOut - more resources

ANNEX G

Notes

Preparation and evaluation in vitro of colloidal lipospheres containing pilocarpine as ion pair

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Abstract

Aqueous dispersions of solid lipospheres containing up to 7.5% pilocarpine as lipophilic ion pairs were submitted to a preliminary evaluation. The lipospheres (diameter 75–85 nm) consisted mainly of stearic acid and egg lecithin; pilocarpine base was incorporated as ion pair with mono-octylphosphate, monodecylphosphate and monohexadecylphosphate. The following parameters were investigated: stability constants (β) and lipophilicity of the ion pairs, size, polydispersity and drug content of the lipospheres, pilocarpine release in vitro. The preparations might constitute a promising vehicle for sustained ocular delivery of pilocarpine.

Keywords: Liposphere; Pilocarpine; Ion pair; Mono-octyl phosphate; Monodecyl phosphate; Monohexadecyl phosphate; Stearic acid; Lecithin

The poor topical bioavailability of pilocarpine (Pi) instilled from conventional preparations is well documented (Schoenwald, 1993). Various approaches aimed at increasing the ocular retention of Pi, and hence its absorption, and/or at improving the transcorneal penetration properties of the drug, have been reported. Substantial improvements have been achieved, to mention only a few examples, with lipophilic prodrugs (Mosher et al., 1987), mucoadhesive complexes (Saettone et al., 1994), nanoparticles (Harmia et al., 1986), pH-sensitive latexes (Ibrahim et al.,

1990), polymeric inserts, including the Ocuser[®] (Urquhart, 1980; Urtti et al., 1985), etc. None of the approaches reported hitherto, however, is exempt from disadvantages, and further investigation in this direction is still deemed useful.

Previous investigations have dealt with the formulation of selected β -blocking agents (timolol, levobunolol) as lipophilic ion pairs (Gallarate et al., 1988, 1993; Gasco et al., 1989; Cavalli et al., 1994). In rabbits, topical administration of timolol as ion pair resulted in a 3–4-fold bioavailability increase with respect to timolol alone (Gasco et al., 1989). Parallel studies were concerned with the preparation of solid colloidal lipospheres as carriers for different drugs (Cavalli et al., 1992, 1993).

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It was postulated, in analogy with a previous study on timolol (Cavalli et al., 1992), that formation of ion pairs would increase the lipophilicity of Pi, whose log P is -3.2 (Leo et al., 1971), and that lipospheres would constitute an optimal carrier for the lipophilic complexes. This preliminary note is concerned with a physico-chemical evaluation of lipophilic ion pairs of Pi, which were incorporated into lipospheres. The in vitro drug release properties of the preparations were investigated, as an essential prerequisite to further studies in vivo.

The preparation of aqueous dispersions containing Pi ion pairs entrapped in lipospheres consisted of two steps: formulation of microemulsion containing stable, lipophilic ion pairs, and preparation of the lipospheres by dispersing the warm microemulsion in cold water. Pilocarpine base (PiB) was prepared from Pi hydrochloride (Sigma, St. Louis, USA). The following sodium salts of monoalkyl esters of phosphoric acid, used as counterions, were prepared as indicated by Brown et al. (1955): mono-octyl phosphate (C-8), monodecyl phosphate (C-10) and monohexadecyl phosphate (C-16).

The stability of the PiB complexes with alkylphosphoric acids was determined by calculating their β stability constants for the overall reaction ($\beta = K_1 K_2$) by potentiometry, as indicated by Irving and Rossotti (1953) and Rossotti and Rossotti (1961). The measurements were carried out in ethanol/water mixtures, and the β values in water were obtained by extrapolation.

For the C-8/Pi complex, the titrations were performed both in water and in a series of ethanol/water mixtures (10, 20, 30, 40, 50, 60 and 70% v/v). The titrations of the C-10 and C-16 Pi complexes were carried out only in the ethanol/water mixtures in which they were soluble. As shown in Table 1, the β values were quite high with C-8, and increased with increasing chain length, thus demonstrating the influence of this parameter on the stability of the ion pairs.

Subsequent efforts were directed at achieving the incorporation of a sufficient amount of PiB in the lipospheres, and at verifying the release characteristics in vitro. For this purpose, three microemulsions, stable at 70°C, and containing 2.1%

Table 1

Stability constants in water (25°C) and apparent partition coefficients (P_{app} , 70°C) of the PiB/alkyl phosphate complexes

Alkyl phosphate	Log β	P_{app}
C-8	11.45	1.4
C-10	12.08	2.5
C-16	13.00	10.8

w/w PiB (A, B, and C), were prepared using C-8, C-10 and C-16, respectively, as counterions. Microemulsions A and B consisted (all percentages w/w) of 7.3% stearic acid (Merck, Darmstadt, Germany) as internal phase, 4.9% purified egg lecithin as surfactant, 5.4% sodium taurodeoxycholate, TDC (Sigma, St. Louis, USA) and 4.4% butanol (Merck, Darmstadt, Germany) as cosurfactants, and 72.5% distilled water as continuous phase. PiB and the alkyl phosphates C-8 or C-10 were added to the microemulsions in the ratio 1:1.5. Microemulsion C consisted of 7.1% stearic acid, 4.8% purified egg lecithin, 6.4% TDC and 5.1% butanol and 71.3% distilled water. The PiB:C-16 molar ratio was 1:1.

Solid lipospheres were obtained by dispersing the warm microemulsions in distilled cold water (2-3°C) under mechanical stirring. The aqueous liposphere suspensions were washed twice with distilled water by ultradiafiltration (Amicon TCF2A, Grace, Danvers USA), and then were freeze-dried (Modulyo freeze dryer, Edwards Crawley, UK).

To evaluate the increase in drug lipophilicity due to the presence of the counterion, the apparent partition coefficients (P_{app}) of PiB between stearic acid and water at pH 6.0 were determined at 70°C, at a pilocarpine/alkyl phosphate ratio of 1:2. After separation of the two phases, the drug concentration in the aqueous phase was determined by HPLC (Perkin Elmer Binary LC Pump 250 liquid chromatograph, Bio-Rad C-8 μ -Bondapack column) as indicated by Durif et al. (1988). The eluent was 5% v/v methanol in 0.05 M phosphate buffer, adjusted to pH 2.5 with triethylamine. The analysis was run at a flow rate of 1.3 ml/min with the detector operating at 214

Table 2
Characteristics of the lipospheres containing different PiB/alkyl phosphate complexes

Lipospheres	Average diameter (nm)	Polydispersity	PiB content (%)
A	85	0.2	5.1
B	75	0.1	7.5
C	70	0.3	5.9

nm (Perkin Elmer LC UV-Visible spectrophotometer).

The P_{app} values (cf. Table 1) were 0.9 for PiB alone, and 1.4, 2.5 and 10.8 for the PiB/C-8, C-10 and C-16 complexes, respectively. These results, which are correlated with the β values of the complexes, are indicative of the increased lipophilicity of the ion pairs, when compared with PiB.

The average diameter of the three types of lipospheres, measured by photon correlation spectroscopy (Zetasizer 2C Malvern, Malvern, UK), was in the range 75 to 85 nm (Table 2). The

amount of PiB incorporated into the lipospheres was determined by HPLC on samples (c.2 mg) of the freeze-dried products, after dissolution in octanol (5.0 ml) and extraction (2×5.0 ml) with pH 2.5 phosphate buffer. As reported in Table 2, the percent PiB incorporated appeared to increase with increasing alkyl chain length from C-8 (5.1%) to C-10 (7.5%), while it was reduced to 5.9% in the case of C-16. The observed decrease was presumably due to the different PiB/alkyl phosphate ratio, which was 1:1 for C-16 and 1:1.5 in the case of the other two counterions.

The release of Pi from the lipospheres was investigated using the method described in a previous paper (Cavalli et al., 1992). As shown in Fig. 1, the amount of PiB diffused through a cellophane membrane (dialysis tubing, Sigma, St. Louis, USA) after 120 min was 9.2 and 7.5% for lipospheres A and B, respectively, and 2.2% for the C lipospheres. Under the same conditions, the percent PiB released from a simple solution was 29.3.

On the basis of the reported preliminary data, the presently described liposphere suspensions containing PiB as ion pair might constitute a promising sustained-release ocular formulation. In vivo tests, now underway, will be the objective of a forthcoming paper.

Acknowledgements

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References

- Brown, D.A., Malkin, T. and Maliphant, G.K., An X-ray examination of long-chain alkyl dihydrogen phosphates and dialkyl hydrogen phosphates and their sodium salt. *J. Chem. Soc.*, (1955) 1584–1588.
- Cavalli, R., Gasco, M.R. and Morel, S., Behaviour of timolol incorporated in lipospheres in the presence of a series of phosphate esters. *STP Pharm. Sci.*, 6 (1992) 514–518.
- Cavalli, R., Caputo, O., and Gasco, M.R., Solid lipospheres of doxorubicine and idarubicin. *Int. J. Pharm.*, 89 (1993) R9–R12.
- Cavalli, R., Serafino, A., Carlotti, M.E. and Gasco M.R., Potentiometric determination of the complexes between a

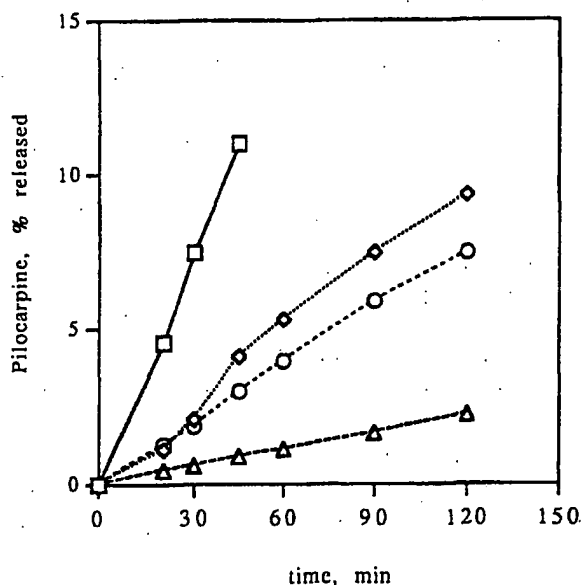


Fig. 1. Percent Pi released from an aqueous solution (□) and from lipospheres containing octyl-phosphate (◇), decyl-phosphate (○) and hexadecyl phosphate (△) ion pairs. The standard deviation of the data was smaller than the size of symbols ($n \geq 3$).

- series of monoalkylphosphoric acid and timolol. *J. Pharm. Sci.*, 83 (1994) 609-610.
- Durif, C., Ribes, M., Kister, G. and Puech, A., Etude comparative du comportement des phases stationnaires en HPLC pour le dosage de la pilocarpine et de ses impurités/produits de dégradation. *Pharm. Acta Helv.*, 63 (1988) 294-298.
- Gallarate, M., Gasco, M.R. and Trotta, M., Influence of octanoic acid on membrane permeability of timolol from solutions and from microemulsions. *Acta Pharm. Technol.*, 34 (1988) 102-105.
- Gallarate, M., Gasco, M.R., Trotta, M., Chetoni, P. and Saettone, M.F., Preparation and evaluation in vitro of solutions and o/w microemulsions containing levobunolol as ion pair. *Int. J. Pharm.*, 100 (1993) 219-225.
- Gasco, M.R., Gallarate, M., Trotta, M., Bauchiero, L., Gremmo, E. and Chiappero, O., Microemulsions as topical delivery vehicles: ocular administration of timolol. *J. Pharm. Biomed. Anal.*, 7 (1989) 433-439.
- Harnia, T., Speiser, P. and Kreuter, J., A solid colloidal drug delivery system for the eye: encapsulation of pilocarpine in nanoparticles. *J. Microencapsul.*, 3 (1986) 3-12.
- Ibrahim, H., Gurny, R., Buri, P., Grove, J., Rozier, A. and Plazonnet, B., Ocular bioavailability of pilocarpine from a phase-transition latex system triggered by pH. *Eur. J. Drug Metab. Pharmacokinet.*, (Special Issue) 7 (1990).
- Irving, H. and Rossotti, H.S., Methods for computing successive stability constants from experimental formation curves. *J. Chem. Soc.*, (1953) 3397-3405.
- Leo, A., Hantsch, C., and Elkins D., Partition coefficients and their use. *Chem. Rev.*, 71 (1971) 525-585.
- Mosher, G.L., Bundgaard, H., Falch, E., Larsen, C., and Mikkelsen, T.J., Ocular bioavailability of pilocarpic acid mono- and diester prodrugs as assessed by miotic activity in the rabbit. *Int. J. Pharm.*, 39 (1987) 113-120.
- Rossotti, F.J.C. and Rossotti, H., *The Determination of Stability Constants*, McGraw-Hill, New York, 1961, pp. 83-118.
- Saettone, M.F., Monti, D., Torracca, M.T. and Chetoni, P., Mucoadhesive ophthalmic vehicles: evaluation of polymeric low-viscosity formulations. *J. Ocul. Pharmacol.*, 10 (1994) 83-92.
- Schoenwald, R.D., Pharmacokinetics in ocular drug delivery. In Edman, P. (Ed.), *Biopharmaceutics of Ocular Drug Delivery*, CRC Press, Boca Raton, FL, 1993, pp. 159-191.
- Urquhart, J., Development of the Ocuser pilocarpine ocular therapeutic systems - A case history in ophthalmic product development. In Robinson, J.R. (Ed.), *Ophthalmic Drug Delivery Systems*, American Pharmaceutical Association, Washington, DC, 1980, pp. 105-116.
- Urtti, A., Periviita, L., Salminen, L. and Juslin, M., Effects of hydrophilicity of polymer matrix on in vitro release rate of pilocarpine and on its miotic activity in rabbit eyes. *Drug Dev. Ind. Pharm.*, 11 (1985) 257-268.

ANNEX H

Inhibition of ceramide biosynthesis preserves photoreceptor structure and function in a mouse model of retinitis pigmentosa

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Edited by Jeremy Nathans, The Johns Hopkins University, Baltimore, MD, and approved September 24, 2010 (received for review June 4, 2010)

Retinitis pigmentosa (RP) is a genetic disease causing progressive apoptotic death of photoreceptors and, ultimately, incurable blindness. Using the retinal degeneration 10 (rd10) mouse model of RP, we investigated the role of ceramide, a proapoptotic sphingolipid, in retinal degeneration. We also tested the possibility that photoreceptor loss can be slowed or blocked by interfering with the ceramide signaling pathway of apoptosis *in vivo*. Retinal ceramide levels increased in rd10 mice during the period of maximum photoreceptor death. Single intraocular injections of myriocin, a powerful inhibitor of serine palmitoyl-CoA transferase, the rate-limiting enzyme of ceramide biosynthesis, lowered retinal ceramide levels to normal values and rescued photoreceptors from apoptotic death. Noninvasive treatment was achieved using eye drops consisting of a suspension of solid lipid nanoparticles loaded with myriocin. Short-term noninvasive treatment lowered retinal ceramide in a manner similar to intraocular injections, indicating that nanoparticles functioned as a vector permitting transcorneal drug administration. Prolonged treatment (10–20 d) with solid lipid nanoparticles increased photoreceptor survival, preserved photoreceptor morphology, and extended the ability of the retina to respond to light as assessed by electroretinography. In conclusion, pharmacological targeting of ceramide biosynthesis slowed the progression of RP in a mouse model, and therefore may represent a therapeutic approach to treating this disease in humans. Transcorneal administration of drugs carried in solid lipid nanoparticles, as experimented in this study, may facilitate continuous, noninvasive treatment of patients with RP and other retinal pathologies.

sphingolipid | apoptosis | electroretinography | morphology

Retinitis pigmentosa (RP), one of the leading causes of blindness worldwide, comprises numerous retinal dystrophies of genetic origin frequently caused by mutations in genes essential to rod photoreceptor function and metabolism, resulting in rod cell death and subsequent night blindness (1). Progressively, cone photoreceptors also die, until all useful sight is lost. Although there is presently no cure for RP, substantial progress has been made to elucidate the genetics and cell biology of these disorders. In particular, rod photoreceptor death in RP is largely thought to occur by apoptosis (2–5), although nonapoptotic mechanisms have also been proposed (5).

Among known proapoptotic cellular messengers, the sphingolipid ceramide is a well-characterized death effector in various experimental models and pathological conditions (6–9). Endogenous cellular levels of ceramide increase after stimulation with different proapoptotic factors that cause acute and chronic disease (10, 11). This increase in ceramide can originate from an activation of *de novo* biosynthesis, a rise in ceramide hydrolysis from sphingomyelin by the action of neutral or acid sphingomyelinases, or a decrease in ceramide metabolism due to glycosylation, phosphorylation, or deacetylation (12). An important role for ceramide-mediated apoptosis in neurodegenerative and neuroinflammatory diseases has now been established (10).

Several lines of evidence suggest that ceramide also mediates apoptosis in retinal photoreceptors (13). First, in *Drosophila* models of RP, genetic manipulation of sphingolipid metabolism has protective effects on retinal morphology and function; protection was achieved both by expressing neutral ceramidase in *Drosophila* eye, to reduce cellular levels of ceramide, and by knocking out one copy of a gene encoding a subunit of serine palmitoyl-CoA transferase (SPT), the enzyme that controls the rate-limiting step of ceramide biosynthesis (14). In humans, a direct genetic link between retinal degeneration and sphingolipid-mediated apoptosis has been established with the discovery that a loss-of-function mutation in CERKL, a gene expressing ceramide kinase-like protein, caused autosomal recessive RP (15, 16), although debate exists on the pathway leading to cell death in individuals with this mutation (17). In rat retinal neuronal primary cultures, oxidative stress increased ceramide levels and caused apoptosis, whereas these effects were blocked by addition of docosahexaenoic acid to stimulate antiapoptotic responses (18). Similarly, in the murine 661W photoreceptor cell line, oxidative stress stimulated acid sphingomyelinase and increased ceramide levels, thereby activating the mitochondrial apoptotic pathway and the caspase cascade (19). Finally, in a rabbit model of retinal apoptosis, ceramide increased after experimental retinal detachment (20). Altogether, these studies document that the accumulation of ceramide is associated with retinal degeneration and suggest that pharmacological interventions altering sphingolipid metabolism may have therapeutic potential.

Effective RP therapies might be implemented on biological models that accurately represent retinal degeneration in humans. Recently, the retinal degeneration 10 (rd10) mouse, with a missense mutation in the β -subunit of the rod-specific phosphodiesterase gene (21), has been shown, through morphological and functional retinal analyses, to be a faithful model of typical human RP (22, 23). In this mutant, photoreceptors begin to die from apoptosis during the third week of life, after the postnatal period of retinal maturation, and photoreceptor death peaks around postnatal day 24 (P24) (22, 23). As in typical human RP, rods die first, whereas cones are lost subsequently. Morphologically, the loss of photoreceptors is accompanied by a progressive thinning of the outer retina and consequent reduction in the number of photoreceptor rows from 12 to 14 on P10 to only 2–3 rows on P30. Functionally, rod-mediated retinal responses to

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light are measurable by electroretinography (ERG) from P14 (the day of eye opening) until P28, although the responses are slow and weak compared with those of wild-type mice (23).

The availability of the rd10 mouse model of RP permitted us to investigate whether pharmacological treatment with myriocin, a selective inhibitor of SPT, could reduce ceramide and thereby exert a protective effect against retinal degeneration. Myriocin, a fungal metabolite discovered for its immunosuppressant properties, is an atypical amino acid with a long hydrophobic tail, structurally similar to sphingosine (24). To our knowledge, myriocin has never been tested for protective effects on retinal degeneration. Here we show that retinas of rd10 mice pups have unusually high levels of ceramide which can be reduced by topical administration of myriocin, concomitantly obtaining both protection of photoreceptors from apoptotic death and maintenance of ERG functional response. Furthermore, we show that noninvasive drug delivery can be achieved using eye drops containing a suspension of myriocin-loaded solid lipid nanoparticles.

Results

To determine whether, in the rd10 mouse model of RP, there are altered levels of retinal ceramide, retinas were obtained between P12 and P30 from rd10 mice pups and from wild-type animals of the same genetic background. Total retinal ceramide, normalized to the amount of inorganic phosphate (Pi) from total phospholipid, was similar in the two groups until P16, after which ceramide content started to increase in rd10 animals but continued to slowly decrease in wild-type mice (Fig. 1A). Significant differences between wild-type and rd10 mice were observed at P21 ($P = 0.045$, t test) and P30 ($P = 0.001$), that is, during the period of maximum retinal degeneration in rd10 mice. To test whether these pathologically high levels could be reduced pharmacologically, we administered myriocin by intraocular injection on P19 followed by ceramide quantification on P21. In 14 of 16 mice, myriocin injection into the right vitreous body reduced retinal ceramide levels below that of the left eye, injected with vehicle alone (Fig. 1B). Overall, myriocin induced a 25.4% reduction in mean ceramide content, from 4.09 pmol/nmol Pi (SD = 1.16) to 3.05 pmol/nmol Pi (SD = 0.97) ($P = 0.011$, paired t test). This treatment was therefore effective in bringing ceramide to levels considered normal for P21. Similar treatment of wild-type mice also reduced ceramide content, by 17.5%, from a mean of 2.00 pmol/nmol Pi (SD = 0.34) to 1.65 pmol/nmol Pi (SD = 0.12) ($P = 0.032$, paired t test).

A possible protective effect of intraocular myriocin injection on photoreceptor survival was investigated by assessing the relative abundance of apoptotic photoreceptors in retinas from myriocin- and DMSO-treated eyes. Isolated retinas were stained with a fluorescent nuclear dye to identify pycnotic (condensed) nuclei and visualized with confocal microscopy. Retinas from myriocin-treated right eyes had fewer intensely stained nuclei than vehicle-

treated left eyes (Fig. 2A and B). Quantitative analysis revealed that 2 d after a single myriocin injection, the number of pycnotic photoreceptor nuclei was reduced by 52.6% ($P = 0.007$, t test) (Fig. 2C). To determine whether these protective cellular effects were associated with a maintenance of retinal function, we measured electrical responses to light by ERG 2 d after a single injection of drug or vehicle. ERG traces from myriocin-treated eyes overlapped with those from DMSO-treated eyes (Fig. S1). Thus, despite a preservation of cellular viability, a single injection of myriocin did not have measurable functional benefits.

Recognizing that continual pharmacological treatment may be necessary to achieve positive effects on retinal function, we topically administered 0.5 nmol myriocin (1 μ L of 3.77 mM solution in DMSO) to the cornea of rd10 mice once daily for 4 d. Microscopic analysis for pycnotic nuclei and ceramide quantification revealed no significant difference between myriocin- and DMSO-treated retinas, suggesting that the drug did not cross the cornea. Therefore, we investigated the possibility of using solid lipid nanoparticles (SLNs) as vehicle to carry the drug across ocular tissues; SLNs are pure lipid particles with a diameter of 40–200 nm. First, we assessed the ability of fluorescently labeled SLNs to reach the interior of the mouse eye. SLNs labeled with the hydrophobic dye *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine (NBD-DPPE) were topically applied to the ocular surface of wild-type mice once daily for 3 d. Confocal microscopy of vertical retinal slices revealed the presence of bright aggregates inside the retina (absent from eyes treated with unlabeled SLNs), mostly in the outer nuclear layer (ONL) and also between the photoreceptors and the retinal pigment epithelium (Fig. 3A). Spectral analysis confirmed that the observed aggregates contained the NBD-DPPE fluorophore (Fig. 3B). Similar observations were obtained for SLNs labeled with coumarin and Nile red. These results demonstrated the feasibility of using SLNs to deliver small, lipophilic molecules across the cornea to the photoreceptors and to the retinal pigment epithelium.

SLNs were subsequently prepared with myriocin. The concentration of the drug in different preparations, considered suitable for use in rd10 mice, ranged from 0.4 to 1.0 mM (mean, 0.6 mM). Myriocin-SLNs were administered topically to both corneas of rd10 mice, three times per day for 3 d starting on P19; additional rd10 mice were treated with control SLNs. In retinas collected on P21, mean values of ceramide were 2.49 pmol/nmol Pi (SD = 0.25) in myriocin-treated animals but 4.19 pmol/nmol Pi (SD = 0.92) in control animals ($P < 0.001$, t test). These results document that myriocin can be effectively administered across the cornea when incorporated into SLNs. However, despite the fact that this treatment reduced retinal ceramide by 40.6% (even more than with a single intraocular injection), still no significant effect was observed on retinal functional responses. Therefore, long-term treatment with myriocin-SLNs was investigated.

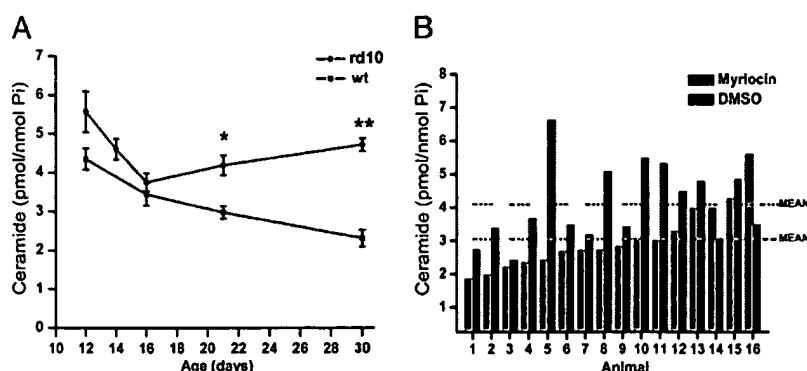


Fig. 1. Ceramide content in mouse retina and effect of myriocin. (A) Time course of endogenous ceramide content in retinas of mice pups during the first month of life: in rd10 mice, ceramide levels begin to increase during the third week of life, in concomitance with retinal degeneration. Values are mean and SE of three to five retinas per data point. * $P = 0.050$; ** $P = 0.001$, t test. (B) Effect of intraocular myriocin injection on retinal ceramide content in rd10 mice. Right eyes were injected on P19 with a single dose of myriocin; left eyes were treated with vehicle. Ceramide content was assessed on P21. Animals are shown in order of increasing ceramide content in myriocin-treated retinas. In 14 of 16 animals, myriocin lowered ceramide content; $P = 0.030$, paired t test.

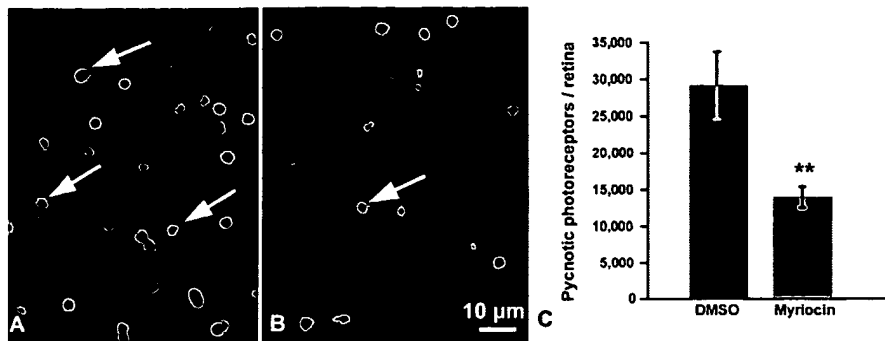


Fig. 2. Intraocular myriocin injection slows photoreceptor loss. (A and B) Fluorescence microscopy of retina whole mounts, fixed and stained with ethidium homodimer, from mice treated with vehicle (A) or myriocin (B) on P19. Myriocin injection was associated with a reduction in the number of pycnotic photoreceptor nuclei on P21. (C) Quantification of pycnotic nuclei per retina. Values are mean (SE) of 16 mice. ** $P = 0.007$, t test.

Starting on P14, rd10 and wild-type (control) mice were treated once daily with myriocin-SLNs or control SLNs. From P21 to P35, different animals underwent ERG testing of retinal function followed by retinal microscopic analysis. Myriocin treatment in wild-type animals had no effect on ERG, which showed the typically large amplitudes of a and b waves (Fig. S2). In both control and myriocin-treated rd10 mice, mean amplitudes of ERG b waves decreased progressively over time in concomitance with retinal degeneration; similar results were observed for absolute values of

mean ERG a waves (Fig. 4). However, absolute values of the mean amplitudes were larger in myriocin-treated than control animals at all time points except P35, when b-wave amplitudes were essentially identical. Significant differences between the two groups were observed for a-wave amplitudes at P30 and P35. These results indicate that continual topical application of myriocin-SLNs can counteract, to a certain extent, the loss of function due to photoreceptor death in rd10 mice.

The maximum effects of myriocin treatment on retinal responses to light of increasing intensity are illustrated in Fig. 5, which shows ERG traces from two rd10 mice pups treated for 10 d (P14–P24) with either myriocin-SLNs or control SLNs. In the myriocin-treated animal, there is higher preservation of both a and b waves of the ERG.

To assess the morphological effects of treatment with myriocin-SLNs on photoreceptors, we counted the number of photoreceptor rows in the outer nuclear layer, considered a more sensitive measure of photoreceptor survival over long periods of treatment than the number of pycnotic nuclei. Microscopic analysis of vertical retinal sections revealed a protective effect of myriocin on the number of photoreceptor rows, seen as a thicker cross-section of the outer nuclear layer (Fig. 6A). This effect was significant at both P24 and P30 (Fig. 6B). The morphology of surviving photoreceptors was preserved as well, as documented by the presence of rhodopsin and cone opsin immunoreactivity in well-organized outer segments of rods and cones, by retention of synaptic terminals of these cells, and by the presence of well-organized dendrites in rod bipolar cells (Fig. S3).

Finally, in a preliminary assessment of safety of long-term treatment, eyes from anesthetized rd10 and wild-type mice treated for 14 d with myriocin-SLNs were examined under a dissection microscope before enucleation for signs of conjunctiva irritation/edema, which were absent. Cataract and corneal opacities could be excluded by the fact that we successfully recorded ERGs. Nuclear staining of retinal sections with ethidium revealed normal retinal histology. Immunohistochemical analyses did not reveal macrophage infiltration (which would have indicated inflammation) nor microglial activation (Fig. S4).

An extension of beneficial effects of myriocin-SLN treatment to retinal cones is anticipated by the morphological preservation of these cells in myriocin-SLN-treated retinas, as well as the recording of a larger cone-driven ERG in rd10 mice treated with myriocin-SLNs for 26 d, compared with control animals (Figs. S3B and S5).

Discussion

This study found that, in the rd10 mouse model of RP, the level of retinal ceramide begins to increase from the third week of life, during the period of maximum photoreceptor loss, whereas in wild-type mice ceramide levels progressively decrease. Single intraocular injections of myriocin, a selective inhibitor of SPT, the rate-limiting enzyme of ceramide biosynthesis, decreased

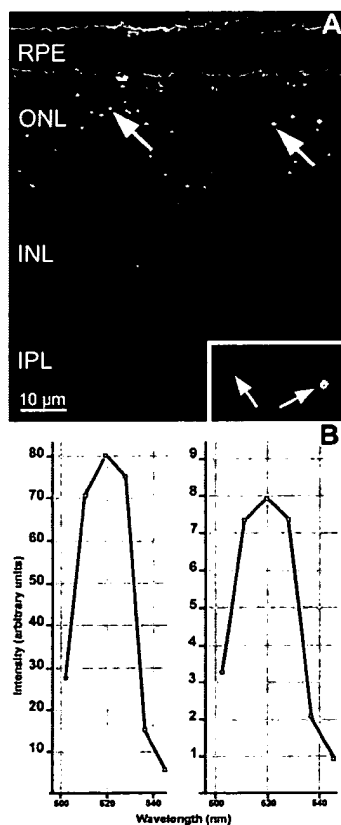


Fig. 3. Topically administered SLNs reach retinal photoreceptors. (A) Fluorescence microscopy of a vertical section of wild-type mouse retina, 3 d after repeated topical application of SLNs labeled with the hydrophobic fluorescent dye NBD-DPPE: bright puncta (arrows) are seen in the outer nuclear layer (ONL) and between photoreceptors and the retinal pigment epithelium (RPE). (Inset) High-magnification of fluorescent puncta of different brightness. INL, inner nuclear layer; IPL, inner plexiform layer. (B) Fluorescence emission spectra from a smear of NBD-DPPE-labeled SLNs (Left) and from puncta in retinal sections of treated mice (Right). The two spectra have the same shape and peak emission at 520 nm, similar to that of NBD-DPPE (39).

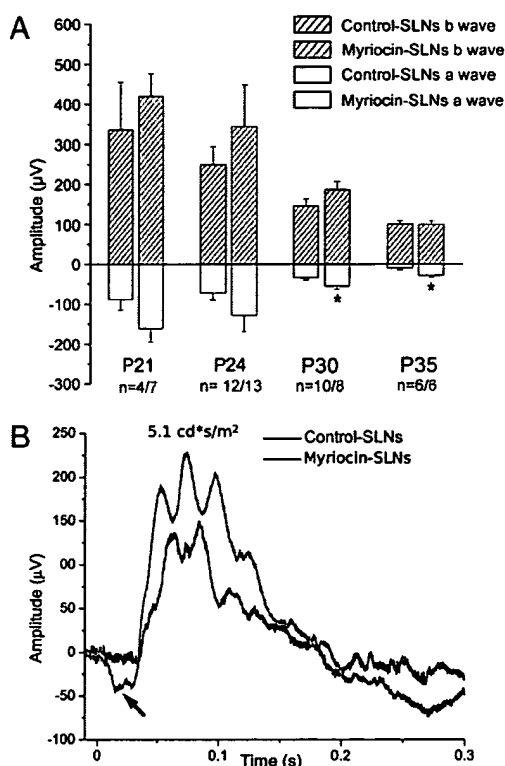


Fig. 4. Effects of long-term administration of myriocin-SLNs on retinal physiology. (A) Amplitudes of ERG a and b waves from eyes of rd10 mice treated with control SLNs or myriocin-SLNs from P14 and exposed to a light flash of 5.1 cd·s/m². Values are mean and SE. Mice treated with myriocin-SLNs (red bars) have larger mean a-wave amplitudes at P30 and P35 (**P* = 0.050, Wilcoxon–Mann–Whitney test). (B) Representative ERG responses to light from two rd10 mice treated for 10 d (from P14 to P24) with myriocin-SLNs (red trace) or control SLNs (black trace). Note the preservation of the a wave following myriocin treatment (arrow).

retinal ceramide and reduced the number of apoptotic photoreceptors in the short term. A functional benefit of myriocin was observed after prolonged daily treatment, achieved by the non-invasive, transcorneal administration of the drug contained in solid lipid nanoparticles. In rd10 mice treated with myriocin-SLNs for 10–20 d, the pathological decrease in photoreceptor number was slowed, photoreceptor morphology was preserved, and the degeneration of retinas was delayed.

The finding that ceramide levels increase in the rd10 mouse in temporal association with the process of photoreceptor demise (22, 23) provides biochemical evidence that this sphingolipid is involved in the neurodegenerative pathology of RP. This result is therefore in accordance with the knowledge derived from genetic studies that human autosomal recessive RP can be caused by loss-of-function mutations in *CERKL*, an enzyme that lowers ceramide content by phosphorylation (15). The results are also in agreement with the increased ceramide levels found in tissues from individuals with other pathologies leading to apoptosis, such as brain tissue from Alzheimer's disease patients (25), and thus provide additional evidence that elevated ceramide is a pathogenetic factor of various diseases. Moreover, the fact that in vivo reduction of retinal ceramide levels slowed disease progression extends to mammals earlier results obtained in *Drosophila* models of RP (14). Therefore, these experiments provide a proof of principle that ceramide regulation represents a relevant therapeutic target in RP as in other pathologies (6, 12, 26–30). Compared with a previous study in *Drosophila* models of RP (14), in which retinal ceramide levels were lowered by genetic manipulation, in this study ceram-

ide levels were lowered pharmacologically. Because noninvasive pharmacological intervention is more easily achieved in humans than gene therapy, the strategy proposed here might become applicable to humans in the long run.

A functional benefit of myriocin was observed only after prolonged treatment but not after a single intraocular administration, despite the fact that both administration methods reduced ceramide levels. The lack of measurable functional effects of single myriocin administrations might be ascribed to the small proportion of photoreceptors rescued from apoptotic death in the short period of study (2 d). Possibly the corresponding effect on the ERG fell below the sensitivity of this functional test.

This study has a few limitations, the most evident being that chronic administration of myriocin rescued a fraction of photoreceptors for a limited time, mostly delaying the inevitable death process in these cells. However, it has to be considered that prolonging the natural evolution of a disease like RP, which characteristically exhibits a slow progression, can nonetheless be beneficial for patients. Another limitation is that we studied a single genetic paradigm of RP, whereas it is known that this disease is genetically heterogeneous. Different mutations leading to photoreceptor demise could activate different pathways of apoptosis (31), within which the sphingolipid cascade could play more or less relevant roles, which need to be studied.

Overall, the results described here are particularly encouraging, considering that the partial loss-of-function rd10 mutation mimics typical RP forms with moderately aggressive phenotype and good retention of retinal architecture (23). These are features that portray human RP patients as likely candidates for gene therapy, in which the defective gene is replaced by a functioning one by means of genetically engineered viral vectors (32). A protective pharmacological approach, based on the non-invasive administration of SPT inhibitors, could prolong the lifespan of photoreceptors in recessive forms of RP, pending gene therapy at a later stage. SPT inhibitors similar to myriocin could therefore contribute to enlarge the panel of bioactive substances already used as neuroprotectants to delay photoreceptor death in this disease (33, 34). In addition, considering that an increment in the rate of rod survival is known to promote a proportionally longer viability of cones, essential for daylight vision, it is possible that the beneficial effects of delaying the sphingolipid-mediated rod demise would propagate to cones as well. Finally, the particular pharmacological strategy used here, based on the employment of lipophilic, tissue-permeant drops, represents a suitable method to deliver molecules to the inner eye noninvasively. Various carriers (i.e., bio- and nonbiodegradable implants, microspheres, nanoparticles, liposomes, and gels) have been tested experimentally to deliver drugs to the inner eye (35). The particular advantages of the SLNs used here are their lack of undesirable effects and their suitability for carrying nonpolar, lipophilic compounds. These features can be exploited for drug delivery in retinal disorders other than RP.

In conclusion, this study demonstrates that pharmacological targeting of ceramide biosynthesis has the potential to slow the progression of RP in a mammalian model and therefore may represent a therapeutic approach to treating this disease in humans. Transcorneal administration of drugs carried in solid lipid nanoparticles, as experimented in this study, may also be developed for human patients with other ocular disorders requiring therapy with lipophilic molecules.

Methods

Animals. rd10 mice (Jackson Laboratories strain B6.CX81-Pde6b^{rd10/j}) (36) and wild-type mice (Jackson Laboratories strain C57BL/6J) were kept in a local facility with water and food ad libitum in a 12-h light/dark cycle with illumination level below 60 lux. Mice were handled according to Italian laws and following the Association for Research in Vision and Ophthalmology

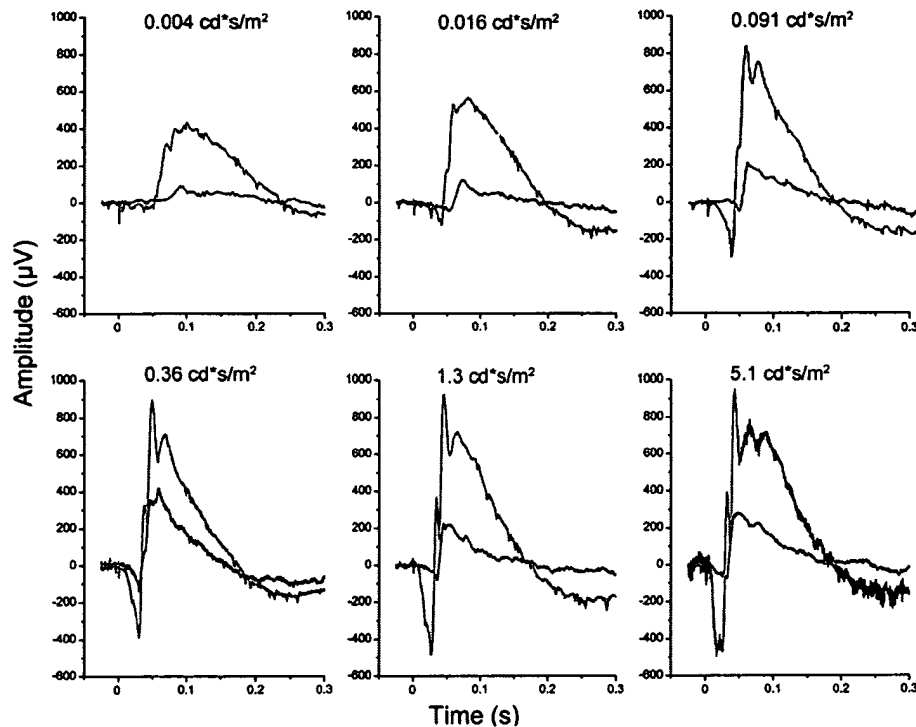


Fig. 5. Effects of myriocin-SLNs on ERG. The best examples of ERG responses to flashes of light of increasing intensity from two rd10 mice age P24. Traces in the panel corresponding to the lowest light intensity are purely rod-driven, whereas the others represent mixed rod-cone responses. Red traces, responses from a mouse treated with myriocin-SLNs; black traces, responses from a mouse treated with control SLNs. In this instance, the SLNs contained the highest myriocin concentration (1 mM) of this study.

(ARVO) statement for the use of animals in research. Protocols were approved by the Italian Ministry of Veterinary Health.

Quantification of Retinal Ceramide. Mice were anesthetized with an i.p. injection of avertin (0.5 g/mL 2,2,2-tribromoethanol in ter-amyl alcohol; 20 μ L/g body weight). Eyes were quickly removed and retinas were detached, placed in oxygenated artificial cerebral-spinal fluid medium, and frozen on dry ice. Retinal lipid was extracted using the Bligh–Dyer method, and total phospholipid was quantified using the Ames method of PI determination. Ceramide was determined by diglyceride kinase assay (*SI Methods*).

Intraocular Injections. Mice were anesthetized as above. Using a dissecting microscope, 500 nL of a 1.88 mM solution of myriocin in DMSO was injected into the right vitreous body using a 10- μ L glass Hamilton syringe driven by an

oil microinjector. Considering that the injected volume is diluted seven- to eightfold within the vitreous body (37), this dosage provides an intraocular concentration of ≈ 0.23 mM myriocin, one order of magnitude higher than that used to inhibit SPT enzymatic activity in single-layer cell-culture studies (38). An identical volume of DMSO vehicle was injected into the left eyes of the same animals.

Fluorescence Microscopy of Retinas for Pycnotic Photoreceptors. Mice were anesthetized as described earlier, and eyes were enucleated and fixed with 4% paraformaldehyde in 0.1 M sodium phosphate (pH 7.2). Retinas were detached and stained with 2 μ M ethidium homodimer 2 (Invitrogen), a fluorescent DNA-intercalating molecule to which fixed tissue is permeable. Whole-mounted retinas were examined under a Leica TCS-SP confocal microscope for the presence of pycnotic (apoptotic) photoreceptor nuclei, brighter than others in the same layer because of the high density of their

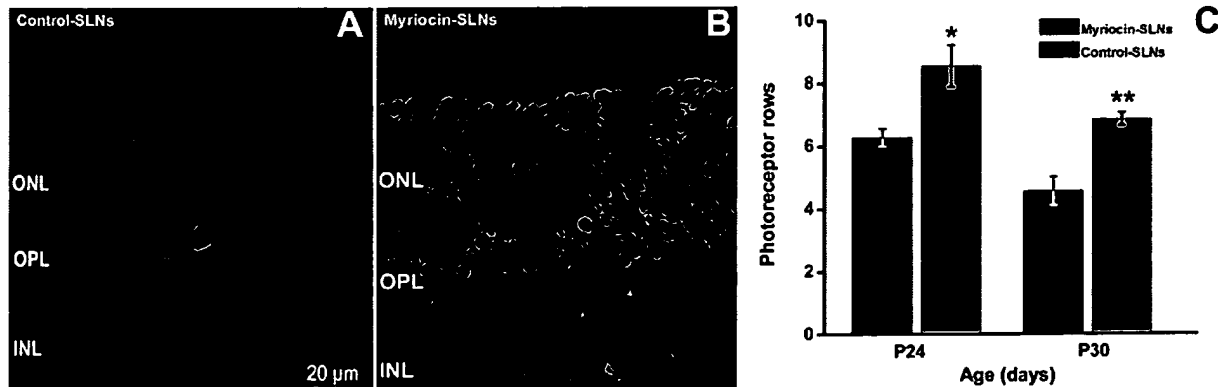


Fig. 6. Effects of myriocin-SLNs on retinal morphology. (A and B) Vertical retinal sections from rd10 mice treated with control SLNs (A) and myriocin-SLNs (B) for 10 d (from P14 to P24). The outer nuclear layer (ONL) of the myriocin-treated retina is thicker because it contains more photoreceptor rows than the control retina. These micrographs are from the same animals whose ERG data are shown in Fig. 4B. INL, inner nuclear layer; OPL, outer plexiform layer. (C) Quantification of photoreceptor rows at P24 and P30 in rd10 mice treated with control SLNs or myriocin-SLNs. Data are mean and SE. * $P = 0.002$, ** $P = 0.003$, t test.

condensed DNA. The ONL, containing the nuclei of photoreceptors, was sampled along the whole z axis. Photoreceptor pycnotic nuclei were counted on projection images of the ONL in fields of $150.6 \times 150.6 \mu\text{m}^2$ (32 fields/retina), spaced at 500- μm intervals along the dorsal-ventral and nasal-temporal retinal meridians. The total number of pycnotic photoreceptors for each retina was calculated by multiplying the average density of pycnotic cells in field images by the corresponding retinal area, measured by low-power light microscopy with an image analyzer (Metamorph 5.0, Universal Imaging Corporation).

Electroretinography. Retinal viability and function were assessed by recording flash electroretinograms as previously described (23); details are given in *SI Methods*. When using mice treated with SLNs, ERG traces were recorded simultaneously from both eyes, identically treated. The possible diffusion of SLNs from one eye to the other made it necessary to use different mice for control and experimental treatments.

Solid Lipid Nanoparticles. Noninvasive, transcorneal treatment of mouse retina was achieved with the use of SLNs, patented by Nanovector srl (*SI Methods*) (40). The concentration of myriocin in SLN preparations was determined by extraction with chloroform:methanol:37% HCl (100:200:1 by volume) followed by TLC on silica gel plates in *t*-butanol:acetic acid:water (3:1:1 by volume). Known amounts of myriocin were loaded on the same TLC plate to generate a standard curve. Separated lipids were visualized by staining with an aqueous solution of 10% CuSO_4 , 8% H_3PO_2 on a hot plate (180 °C) for 3–6 min. Myriocin spots were quantified by densitometry (Gel Doc 2000 and Quantity One software; Bio-Rad).

Topical Administration of SLNs. To determine whether lipophilic molecules contained in SLNs penetrate the inner eye, wild-type mice were treated three times per day with 1 μL SLNs labeled with coumarin, Nile red, or NBD-DPPE; unlabeled SLNs served as control. After 3 d of treatment, vertical retina sections were examined by fluorescence confocal microscopy for the presence and localization of the fluorophore within the retina. To determine

whether the observed fluorescence corresponded to the fluorophore administered topically, the emission spectrum from retinal sections was compared with that of a smear of the SLNs on a microscope slide by fluorescent spectral analysis using the confocal microscope's 488-nm laser source. For pharmacological treatment, rd10 mice (66 animals from 10 litters) and wild-type mice ($n = 12$) were administered once daily with either myriocin-SLNs or control SLNs in both eyes. Each treatment consisted of a double dosage, within a 15-min interval, of 750–1,000 nL/eye (larger volumes were used in older animals). Treatment started on P14 (eye opening) and continued until analysis between P21 and P35.

Fluorescence Microscopy on Retinal Vertical Sections. Retinas from rd10 and wild-type mice that had been treated with SLNs were harvested for morphological analysis. Photoreceptor survival was assessed by counting nuclei in the ONL. Photoreceptor morphology was assessed by immunohistochemistry (*SI Methods*).

Statistical Analysis. Data were compared by two-tailed unpaired or paired *t* tests or with the Wilcoxon–Mann–Whitney test when the distribution of data was not normal, as assessed by analysis with SigmaStat v. 3.1 (Systat). Statistical analysis was performed with Origin v. 7.0 (OriginLab) and SigmaStat v. 3.1 software packages for Windows XP. A *P* value <0.05 was considered significant.

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- Hartong DT, Berson EL, Dryja TP (2006) Retinitis pigmentosa. *Lancet* 368:1795–1809.
- Corrochano S, et al. (2008) Attenuation of vision loss and delay in apoptosis of photoreceptors induced by proinsulin in a mouse model of retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 49:4188–4194.
- Cottet S, Schorderet DF (2009) Mechanisms of apoptosis in retinitis pigmentosa. *Curr Mol Med* 9:375–383.
- Portera-Cailliau C, Sung CH, Nathans J, Adler R (1994) Apoptotic photoreceptor cell death in mouse models of retinitis pigmentosa. *Proc Natl Acad Sci USA* 91:974–978.
- Sancho-Pelluz J, et al. (2008) Photoreceptor cell death mechanisms in inherited retinal degeneration. *Mol Neurobiol* 38:253–269.
- Billich A, Baumrucker T (2008) Sphingolipid metabolizing enzymes as novel therapeutic targets. *Subcell Biochem* 49:487–522.
- Deng X, et al. (2008) Ceramide biogenesis is required for radiation-induced apoptosis in the germ line of *C. elegans*. *Science* 322:110–115.
- Hannun YA, Obeid LM (2008) Principles of bioactive lipid signalling: Lessons from sphingolipids. *Nat Rev Mol Cell Biol* 9:139–150.
- Morales A, Lee H, Goñi FM, Kolesnick R, Fernandez-Checa JC (2007) Sphingolipids and cell death. *Apoptosis* 12:923–939.
- Jana A, Hogan EL, Pahan K (2009) Ceramide and neurodegeneration: Susceptibility of neurons and oligodendrocytes to cell damage and death. *J Neurol Sci* 278:5–15.
- Toman RE, Spiegel S, Faden AI (2000) Role of ceramide in neuronal cell death and differentiation. *J Neurotrauma* 17:891–898.
- Kitatani K, Idkowiak-Baldys J, Hannun YA (2008) The sphingolipid salvage pathway in ceramide metabolism and signaling. *Cell Signal* 20:1010–1018.
- Rotstein NP, Miranda GE, Abraham CE, German OL (2010) Regulating survival and development in the retina: Key roles for simple sphingolipids. *J Lipid Res* 51:1247–1262.
- Acharya U, et al. (2003) Modulating sphingolipid biosynthetic pathway rescues photoreceptor degeneration. *Science* 299:1740–1743.
- Avila-Fernandez A, et al. (2008) CERKL mutations and associated phenotypes in seven Spanish families with autosomal recessive retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 49:2709–2713.
- Tuson M, Marfany G, González-Duarte R (2004) Mutation of CERKL, a novel human ceramide kinase gene, causes autosomal recessive retinitis pigmentosa (RP26). *Am J Hum Genet* 74:128–138.
- Graf C, Niwa S, Müller M, Kinzel B, Bornancin F (2008) Wild-type levels of ceramide and ceramide-1-phosphate in the retina of ceramide kinase-like-deficient mice. *Biochem Biophys Res Commun* 373:159–163.
- German OL, Miranda GE, Abraham CE, Rotstein NP (2006) Ceramide is a mediator of apoptosis in retina photoreceptors. *Invest Ophthalmol Vis Sci* 47:1658–1668.
- Sanvicens N, Cotter TG (2006) Ceramide is the key mediator of oxidative stress-induced apoptosis in retinal photoreceptor cells. *J Neurochem* 98:1432–1444.
- Ranty ML, et al. (2009) Ceramide production associated with retinal apoptosis after retinal detachment. *Graefes Arch Clin Exp Ophthalmol* 247:215–224.
- Chang B, et al. (2007) Two mouse retinal degenerations caused by missense mutations in the β -subunit of rod cGMP phosphodiesterase gene. *Vision Res* 47:624–633.
- Barhoum R, et al. (2008) Functional and structural modifications during retinal degeneration in the rd10 mouse. *Neuroscience* 155:698–713.
- Gargini C, Terzibasi E, Mazzoni F, Strettoi E (2007) Retinal organization in the retinal degeneration 10 (rd10) mutant mouse: A morphological and ERG study. *J Comp Neurol* 500:222–238.
- Miyake Y, Kozutsumi Y, Nakamura S, Fujita T, Kawasaki T (1995) Serine palmitoyltransferase is the primary target of a sphingosine-like immunosuppressant, ISP-1/myriocin. *Biochem Biophys Res Commun* 211:396–403.
- He B, Lu N, Zhou Z (2009) Cellular and nuclear degradation during apoptosis. *Curr Opin Cell Biol* 21:900–912.
- Claus RA, Dorer MJ, Bunck AC, Deigner HP (2009) Inhibition of sphingomyelin hydrolysis: Targeting the lipid mediator ceramide as a key regulator of cellular fate. *Curr Med Chem* 16:1978–2000.
- Liu X, et al. (2008) Acid ceramidase inhibition: A novel target for cancer therapy. *Front Biosci* 13:2293–2298.
- McEachern KA, et al. (2007) A specific and potent inhibitor of glucosylceramide synthase for substrate inhibition therapy of Gaucher disease. *Mol Genet Metab* 91:259–267.
- Ozbayraktar FB, Ulgen KO (2009) Molecular facets of sphingolipids: Mediators of diseases. *Biotechnol J* 4:1028–1041.
- Schenck M, Carpinteiro A, Grassmé H, Lang F, Gulbins E (2007) Ceramide: Physiological and pathophysiological aspects. *Arch Biochem Biophys* 462:171–175.
- Marigo V (2007) Programmed cell death in retinal degeneration: Targeting apoptosis in photoreceptors as potential therapy for retinal degeneration. *Cell Cycle* 6:652–655.
- Cai X, Conley SM, Naash MI (2009) RPE65: Role in the visual cycle, human retinal disease, and gene therapy. *Ophthalmic Genet* 30:57–62.
- Komeima K, Rogers BS, Campochiaro PA (2007) Antioxidants slow photoreceptor cell death in mouse models of retinitis pigmentosa. *J Cell Physiol* 213:809–815.
- Sieving PA, et al. (2006) Ciliary neurotrophic factor (CNTF) for human retinal degeneration: Phase I trial of CNTF delivered by encapsulated cell intraocular implants. *Proc Natl Acad Sci USA* 103:3896–3901.
- Janoria KG, Gunda S, Boddu SH, Mitra AK (2007) Novel approaches to retinal drug delivery. *Expert Opin Drug Deliv* 4:371–388.
- Chang B, et al. (2002) Retinal degeneration mutants in the mouse. *Vision Res* 42:517–525.
- Yu DY, Cringle SJ (2006) Oxygen distribution in the mouse retina. *Invest Ophthalmol Vis Sci* 47:1109–1112.
- Scarlatti F, et al. (2003) Resveratrol induces growth inhibition and apoptosis in metastatic breast cancer cells via de novo ceramide signaling. *FASEB J* 17:2339–2341.
- Chattopadhyay A, London E (1988) Spectroscopic and ionization properties of *N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-labeled lipids in model membranes. *Biochim Biophys Acta* 938:24–34.
- Gasco MR, Gasco P (2007) Nanovector. *Nanomedicine* 2:955–960.

ANNEX I

Supporting Information

Strettoi et al. 10.1073/pnas.1007644107

SI Methods

Diglyceride Kinase Assay for Ceramide Determination. Phospholipid (30 nmol) was dried and redissolved in 20 μ L β -octyl glucoside-dioleoyl-phosphatidylglycerol micelles (1). This was followed by addition of 50 μ L buffer (0.1 M imidazole, pH 6.6, 0.1 M LiCl, 25 mM MgCl_2 , 2 mM EGTA), 0.2 μ L 1 M DTT, 7 μ L recombinant diacylglycerol kinase (Calbiochem-Merck; 1 μ g/ μ L), and 20 μ L 1 mM diethylene triamine pentaacetic acid in 10 mM imidazole (pH 6.6). The reaction was started by addition of 10 μ L ATP solution (1 mM ATP and 1.3 μ Ci [γ - 32 P]ATP at 3 Ci/ μ mol). After 45 min at 25 $^{\circ}\text{C}$, lipid was extracted, dried, resuspended in chloroform:methanol (1:1 by volume), and analyzed along with reference standards [ceramide, brain (product no. 860052), and 1,2-dioleoyl-*sn*-glycerol; Avant Polar Lipids] by TLC on silica gel 60 plates (Whatman) in chloroform:acetone:methanol:acetic acid:water (10:4:3:2:1, by volume). Radiolabeled ceramide-1-phosphate spots were visualized by autoradiography, scraped, and counted by liquid scintillation. The amount of ceramide per retina was normalized to that of total phospholipid and expressed in units of pmol/nmol inorganic phosphate; data from three to five mice are expressed as mean and SE.

Electroretinography. Mice were dark-adapted overnight and anesthetized with an i.p. injection of ketamine (2.5 μ g/g body weight) and xylazine (0.3 μ L/g body weight). Body temperature was maintained at 37 $^{\circ}\text{C}$. Pupils were dilated by administration of tropicamide (1%) eye drops, and the cornea was kept moist with a methylcellulose solution. For electroretinography (ERG), an electronic flash unit generated a light stimulus of 492 nm whose energy decayed with a τ of 1.7 ms. Full-field stimulation was achieved using a Ganzfeld sphere, and flash intensities were attenuated by neutral-density filters. Mice were subjected to six different flash intensities, each repeated five times, with an interstimulus interval that ranged from 60 s for dim light to 5 min for the brightest flashes. The flash luminance was measured at the corneal plane in photometric units ($\text{cd}\cdot\text{s}/\text{m}^2$) using a Minolta CS100 photometer with a scotopic filter. ERG signals were recorded using coiled gold corneal electrodes. Responses were differentially amplified, band-pass-filtered at 0.3–500 Hz, digitized at 0.25- to 0.5-ms intervals, and stored on disk for processing. Five ERG traces at each flash luminance were averaged before measurements of a- and b-wave amplitudes. The amplitude of the a wave was taken as the difference between baseline and the lowest value, whereas the amplitude of the b wave was measured from the trough of the a wave to the peak of the b wave. The responses to the brightest flashes include mixed rod and cone components. Isolated cone components were obtained by superimposing test flashes on a background of saturating intensity for rods (30 $\text{cd}\cdot\text{s}/\text{m}^2$).

Solid Lipid Nanoparticles. Noninvasive treatment of retinas was achieved with the use of solid lipid nanoparticles (SLNs), pure lipid particles with a diameter of 40–200 nm containing a fatty acid core wrapped in a layer of phospholipids (2). For feasibility testing, SLNs were loaded with the fluorescent dyes coumarin (Acros Organics), Nile red (Sigma-Aldrich), or *N*-(7-nitro-2-1,3-benzoxadiazol-4-yl)-1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine

(NBD-DPPE; NOF Corporation) at a concentration of 0.01–0.02% (wt/wt). For experimental testing, SLNs were loaded with 0.15% (wt/wt) myriocin (Sigma-Aldrich). Briefly, an oil phase composed of melted stearic acid and Epikuron 200 (96% phosphatidylcholine; Cargill) and a water phase consisting of taurocholic acid sodium salt (Prodotti Chimici Alimentari) and water were combined in a warm microemulsion; fluorescent dyes or myriocin were then added (2). Butylated hydroxyanisole and D,L- α -tocopherol were included to prevent oxidation. The microemulsion was dispersed in cold water, resulting in the solidification of nanodrops which were then washed by tangential filtration (Amicon 8010 stirred ultrafiltration cell; Millipore). SLN preparations were sterilized by filtration through a 0.2- μ m filter before use.

Fluorescence Microscopy on Retinal Vertical Sections. After ERG studies of mice treated with myriocin-SLNs or control SLNs, eyes were removed, fixed for 1 h in 4% paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4), infiltrated with 30% sucrose in the same buffer, and frozen at -20°C on a cryostat stage (Leica). Vertical sections of 14 μ m were cut, collected on glass microscope slides, and stained with ethidium homodimer 2 before examination by confocal microscopy. Because these mice had been treated for 7–21 d, the effects of myriocin on photoreceptor apoptosis could not be accurately determined by staining for pycnotic nuclei, as the acute phase of photoreceptor degeneration had already passed (3). Therefore, we estimated the number of surviving photoreceptors by counting the rows of nuclei in the outer nuclear layer on high-resolution images of vertical sections obtained from both central and peripheral retinal areas (10 images/retina, each covering a linear retinal extension of 250 μ m). Mean values per retina were averaged per group and expressed as mean and SE.

Photoreceptor morphology and retinal histology were studied by immunocytochemistry and confocal microscopy on retinal sections as explained in ref. 3. Primary antibodies used were anti-rhodopsin (mouse monoclonal; Sigma), anti-cone-specific opsins (rabbit polyclonal; Chemicon), anti-PSD-95 (mouse monoclonal; Chemicon), and anti-protein kinase C (PKC) α (rabbit polyclonal; Sigma). All of the primary antibodies were used at 1:1,000 dilution and revealed with Oregon green-conjugated secondary antibodies. Retinal sections were counterstained with ethidium homodimer to reveal nuclei. Macrophage infiltration (a sign of local inflammation) was assessed on retinal sections from wild-type and rd10 mice treated with topical nanospheres using anti-F4/80 antibodies (rabbit polyclonal; Santa Cruz Biotechnology; used at a dilution of 1:50). Positive controls used for these antibodies were retinal whole mounts from rd10 mouse eyes which had received a massive intraocular injection and thus mechanically damaged and mouse hippocampus, previously treated with kainic acid and thus infiltrated with macrophages. Sections from retinal samples treated with topical nanospheres were also stained with fluorescent *Griffonia simplicifolia* lectin (Sigma; 1:500) to visualize blood vessels and microglia, with the rationale of detecting possible activation of retinal microglial cells following nanosphere administration.

1. Yu DY, Cringle SJ (2006) Oxygen distribution in the mouse retina. *Invest Ophthalmol Vis Sci* 47:1109–1112.

2. Gasco MR, Gasco P (2007) Nanovector. *Nanomedicine* 6:955–960.

3. Gargini C, Terzibasi E, Mazzoni F, Strettoi E (2007) Retinal organization in the retinal degeneration 10 (rd10) mutant mouse: a morphological and ERG study. *J Comp Neurol* 505:222–238.

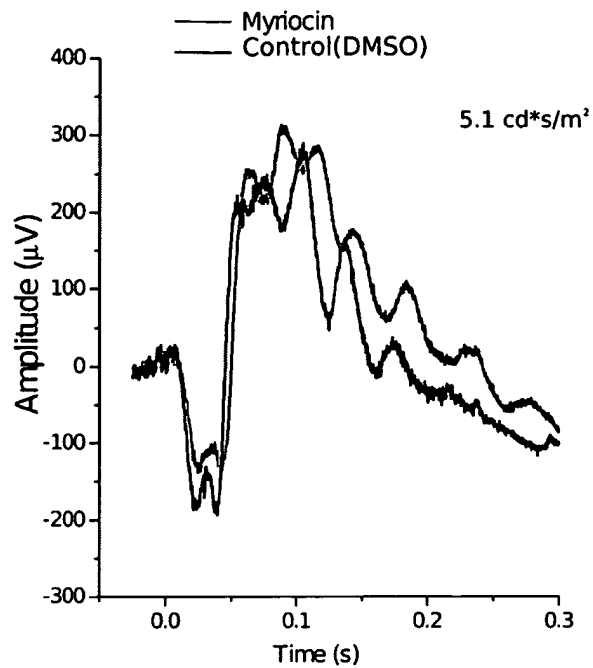


Fig. S1. Lack of functional effects of a single myriocin administration. Representative electroretinograms from eyes treated with myriocin (red trace) or vehicle (black trace). A single intraocular injection of myriocin did not have measurable protective effects on retinal function after 2 d, as shown by similar amplitudes and kinetics of a and b waves in the two experimental conditions.

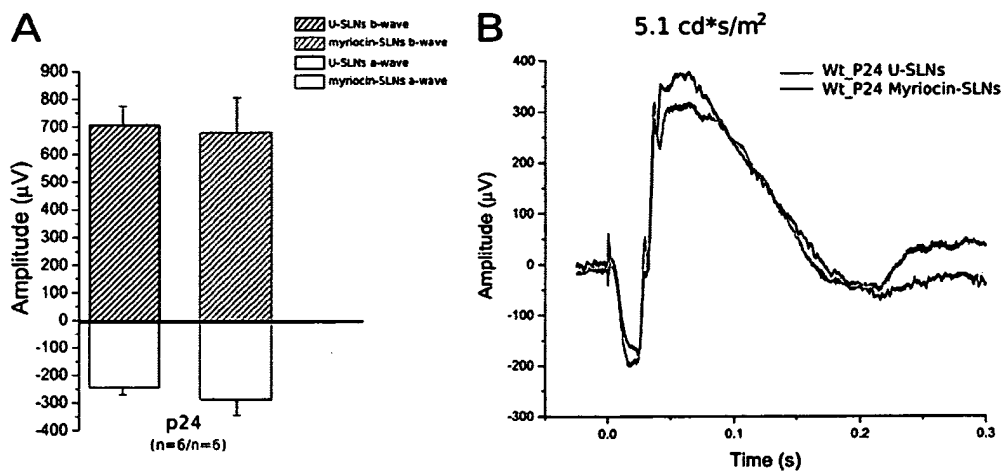


Fig. S2. Lack of functional effects of myriocin treatment on wild-type mouse retinas. (A) Average of six treated and untreated mice. Treatment with myriocin did not induce significant changes in either a-wave or b-wave amplitudes. (B) Representative ERG traces obtained from a wild-type mouse after prolonged administration of myriocin-SLNs (red traces). No differences were observed with respect to littermate animals treated with control SLNs (black traces). Treatment started at postnatal day 14 (P14) and ended at P24, the time of ERG recording.

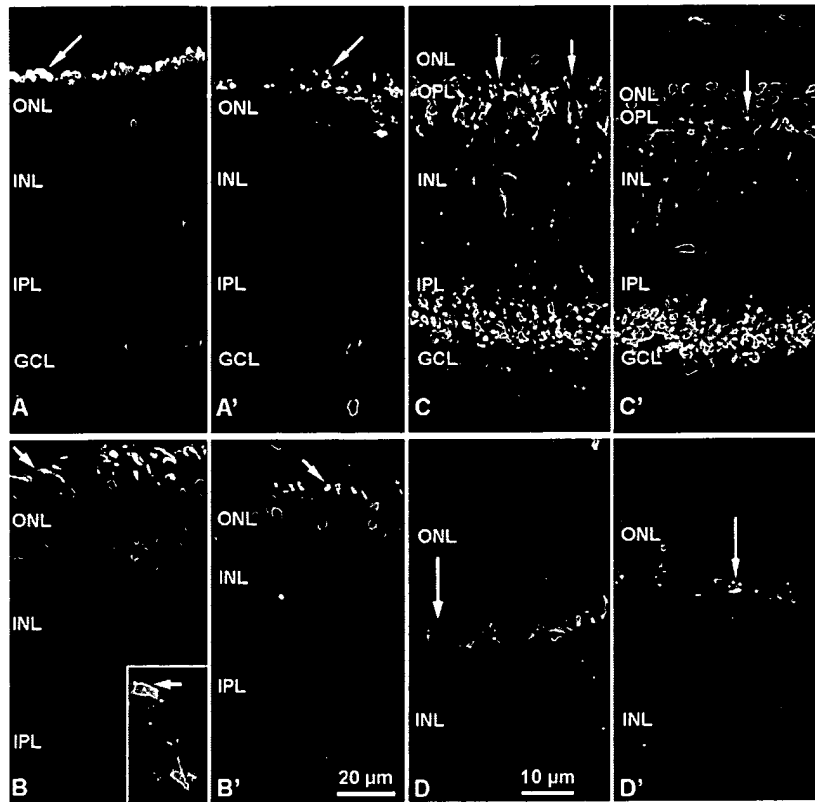


Fig. S3. rd10 retinal morphological preservation upon chronic treatment with myriocin-SLNs. Vertical sections of rd10 retinas from mice treated with myriocin-SLNs (A–D) and from control rd10 mice treated with unloaded SLNs (A'–D'). Age: P35. (A and A') Rhodopsin antibody staining (green signal). Rod outer segments (arrows) are shortened but still continuous in A, whereas they are scant and poorly organized in A'. In these and the other panels, the red signal represents nuclear counterstaining with ethidium. (B and B') Cone opsin antibody staining (green signal). Cones retain elongated and well-organized outer segments in B but are shortened and sparser in B' (arrows). (Inset) High magnification of a cone entirely labeled from a myriocin-treated retina. (C and C') Rod bipolar cells stained for PKC α (green signal) display normal morphology with well-organized dendritic trees (arrows) in the outer plexiform layer of C; their dendrites have regressed considerably in control rd10 mice (C'). The scale bar in B' holds for A–C'. (D and D') Synaptic terminals of rods and cones labeled with anti-PSD-95 antibodies (green signal). The arrow points to a cluster of spherules in the outer plexiform layer; note the aberrant morphology of these terminals in the control preparation (D'). GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; ONL, outer nuclear layer; OPL, outer plexiform layer.

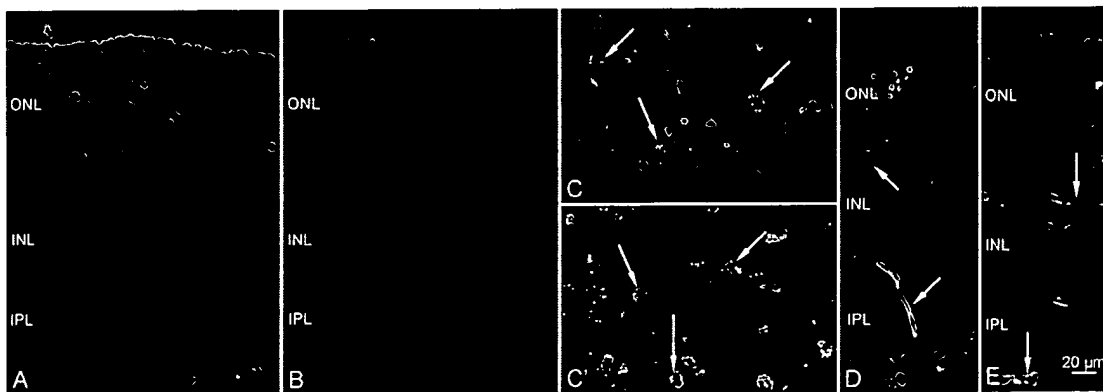


Fig. S4. Absence of adverse effects on retinal morphology of prolonged myriocin treatment. Vertical retinal sections from rd10 (A) and wild-type (B) mice age P28, treated for 14 d with myriocin-SLNs. Staining for the macrophage-specific marker F480 (green) did not provide evidence of infiltration of macrophages, indicative of inflammatory response. The overall retinal structure is normal, as shown by nuclear counterstaining with ethidium (red signal). (C and C') Positive controls of the F480 antibody used in A and B. (C) Retinal whole mount from an rd10 mouse which received an invasive intraocular injection causing retinal mechanical damage and thus macrophage activation. The focus is on the ganglion cell layer. Arrows point to F480-positive macrophages (green staining). (C') Section of a mouse hippocampus previously treated with an injection of kainic acid. Macrophages, labeled with F480 (arrows, green staining), infiltrate the tissue. (D and E) *G. simplicifolia* lectin staining (green signal) of retinal sections from rd10 (D) and wild-type (E) mice treated with myriocin-SLNs. As expected, retinal blood vessels (arrows) are labeled, whereas the staining does not reveal activated microglial cells.

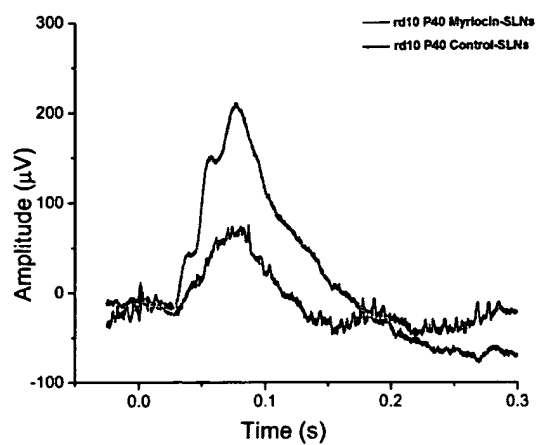


Fig. 55. Cone-driven ERG responses from rd10 mice (red trace) treated with myriocin-SLNs or control SLNs (black trace). Animals were age P40 and treatment had initiated at P14. This photopic ERG was obtained upon stimulation with a strong light flash ($87 \text{ cd}\cdot\text{s}/\text{m}^2$) superimposed on a rod-saturating adaptive background of $30 \text{ cd}/\text{m}^2$.

PATENT
ATTORNEY DOCKET NO. 209,127

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s):	Gasco et al.	Confirmation No.:	4539
Serial No.:	10/533,512	Art Unit:	1612
Filed:	May 02, 2005	Examiner:	Huang, G. G.
Title:	"Pharmaceutical compositions suitable for the treatment of ophthalmic diseases"		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.132

I, Gian Paolo Zara, being duly sworn depose and say that:

1. I am an Italian citizen residing at: Turin (IT).
2. I am familiar with the English language.
3. I graduated in: Medicine (1977) at the University of Turin.
4. I currently am a Researcher, particularly a Clinical Pharmacologist, at the Department of Pharmacological Anatomy and Legal Medicine of the University of Turin (IT). (see enclosed my CV)
5. I am author of more than 180 scientific publications.
6. I am one of the inventors of the current US patent application.

Experimental section

I was contacted in 2002 by Prof. MR Gasco for evaluating the pharmacokinetic and biodistribution of drugs incorporated in solid lipid nanoparticles (SLN), with particular regard for the drug penetration rate into the rabbit eyes.

I therefore personally organized the experimental pharmacological studies and the animal model. The results of said studies have been reported in the current patent application.

It was surprisingly and unexpectedly found that, by comparing SLN-drug dispersions according to the invention and commercial products, the former reached the retina, also in amounts advantageously high, i.e. effective amounts, both through intravenous administration and topical ocular administration.

Particularly, Examples 2 and 3 reported the results of SLN-gentamicin intravenous administration were compared to Gentomil® intravenous administration, as follows:

Concentration of Gentamicin		SLN-gentamicin	Gentomil®
Injected dose: 1.5 mg/Kg		(1 hour after administration)	
in the aqueous fluid	right eye	300 ng/100 µl	50 ng/100 µl
	left eye	326 ng/100 µl	56 ng/100 µl
in the vitreous fluid	right eye	499 ng/100 µl	3.5 ng/100 µl
	left eye	531 ng/100 µl	2.5 ng/100 µl
in the retina	right eye	1.225 ng/100 µl	non perceptible
	left eye	1.365 ng/100 µl	non perceptible
Injected dose: 2 mg/Kg		(3 hours after administration)	
in the aqueous fluid	right eye	244 ng/100 µl	40 ng/100 µl
	left eye	120 ng/100 µl	36 ng/100 µl

in the vitreous fluid	right eye	126 ng/100 μ l	non perceptible
	left eye	157 ng/100 μ l	non perceptible
in the retina	right eye	99.5 ng/100 μ l	non perceptible
	left eye	84 ng/100 μ l	non perceptible

It can be immediately understood that, not only Gentamicin has been proved to reach the tissues of the posterior segment of the eye, but also that the amount of drug there delivered is surprisingly high, especially when considering the absolutely unsatisfactory results of the compared commercial product carrying the same drug.

Additionally, Examples 4 and 5 reported the results of SLN-gentamicin topical ocular administration were compared to Genticol® topical ocular administration, as follows:

Concentration of Gentamicin	SLN-gentamicin	Genticol®
Topical dose: 2 mg/ml	(1 hour after administration)	
in the aqueous fluid	10 μ g/100 μ l	5 μ g/100 μ l
in the vitreous fluid	2.76 μ g/100 μ l	not perceptible
in the retina	890 ng/100 μ l	non perceptible
Topical dose: 200 μ l/ml	(1 hour after administration)	
in the aqueous fluid	35 ng/100 μ l	16 ng/100 μ l
in the vitreous fluid	7.84 ng/100 μ l	non perceptible
in the retina	5.4 ng/100 μ l	non perceptible

It can be immediately understood that, also in the case of topical ocular administration, not only Gentamicin has been proved to reach the tissues of the posterior segment of the eye, but also that the amount of drug there delivered is surprisingly high, especially when considering the absolutely unsatisfactory results of the compared commercial product carrying the same drug.

It should be noted that these results were absolutely unexpected at the time the invention was made, since no indications in prior art was available to the skilled person to believe that SLN could pass all the eye tissues as well as the blood retinal barrier, so reaching the retina and the choroid.

Additionally, in the paper of Cavalli et al. the aim of the study expressly was to evaluate the drug concentration in the anterior chamber of the eye. In fact, at that time no drug carriers were known able to successfully reach the retina and consequently there were no expectation of success in the further studying SLNs for treating the posterior segment of the eye. This is the reason why the method, as reported in the current patent application, demonstrating the possibility of treating the posterior segment of the eye through a non-invasive way, i.e. by using SLNs as drug carrier, was an absolutely great and unexpected scientific and technical result.

As far as the document "Amselem" is concerned, it should be considered that in the example 17, the study is performed in normal rabbits without any ocular pathology, so it is impossible and scientifically unacceptable to speculate that a drug reducing eye pressure is effective in lowering the ocular pressure in glaucoma disease, even considering that the emulsomes are different from SLN both chemically as such and in term of drug delivery, and that only a type of glaucoma is associated to high IOP.

**** ** *

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patents issued thereon.

Date: January 24, 2011

A handwritten signature in black ink, appearing to read 'Gian Paolo Zara', written in a cursive style.

[Signature: Gian Paolo Zara]

CURRICULUM VITAE

ZARA, GIAN PAOLO

Born in Sassari on May 5, 1952

Graduated cum laude in Medicine at the University of Turin (1977)

Specialized cum laude in General Surgery at the University of Turin (1984)

Titles

From 1/Feb/1978 to 31/Dec/1980

Doctor at the Institute of Surgical Anatomy of the University of Turin

From 1/Jan/1981 to 13/Sep/1992

Specialising Doctor at the Institute of Surgical Anatomy of the University of Turin

From 1/Nov/1982 to 3/Mar/1984

Research Fellow at the Surgical and Gastrointestinal Research Unit of the London Hospital Medical College

Dal 1/11/1985 al 31/11/1987

Research Fellow at the Surgical and Gastro-intestinal Research Unit of the London Hospital Medical College

6/Jul/1988: Achievement of compliance as Head Physician in General Surgery

26/Sep/1990: Achievement of compliance as Research Fellow at the Faculty of Medicine of the University of Turin.

14/Sep/1992 Achievement of compliance as Researcher at the Faculty of Medicine of the University of Turin.

1/11/1995: in force at the Department of Pharmacological Anatomy and Legal Medicine of the University of Turin

From 1994 to date: Lecturer in Pharmacology at the Nursing Science School of the University of Turin

From 1998 to date: Lecturer in Pharmacology at the Dental Science School of the University of Turin

From 1997 to date: Lecturer in Pharmacology at the Obstetrics Science School of the University of Turin

From 1994 to 1996: Lecturer in Pharmacology at the Anaesthetics Science School of the University of Turin

Memberships

From June 1990: member of the European Society for Intestinal Motility

From November 1987: member of the Royal Society of Medicine of London

From July 1990: member of the American Medical Informatics Association

From June 1999: member of the Italian Pharmacological Society

SCIENTIFIC PUBLICATIONS

1) Zara G.P., Thompson H.H., Pilot M.A., Ritchie H.D.

Effects of Erythromycin on gastrointestinal tract motility

Journal of Antimicrobial Chemotherapy 1985, 16:(Suppl.A) 175-179

2) Pilot M.A., Thompson H.H., Zara G.P.

Effect of 5-hydroxytryptamine on canine intestinal motility during fasting

J.Physiol. 1983, 343:88-89P

3) Pilot M.A., Thompson H.H., Zara G.P.

Mechanism of action of 5-hydroxytryptamine on canine intestinal motility

J.Physiol 1984, 354:41P

4) Pilot M.A., Ritchie H.D., Thompson H.H., Zara G.P.

Alterations in gastrointestinal motility associated with erythromycin

Br.J.Pharmac. 1984, 81:168P

5) Davidson H.I., G.P. Zara G.P., Pilot M.A., Thompson H.H.

Pre-ganglionic transmission of serotonergic activity in the digestive tract

Abstract Tenth International Symposium on Gastrointestinal Motility. Rochester

U.S.A. September 8-11 1985. Dig.Dis.Sci. 30:766 (1989)

6) Masenti E., Mioli P., Gaglia P., Zara G.P.

Patologia del giunto gastro-esofageo: ricerche manometriche e risultati clinici

Giornale dell'Accademia di Medicina di Torino 1979

7) Sartoris A., Giordano C., Di Fortunato V., Tabaro G., Mioli P., Zara G.P.,

Potenzoni F., Orecchia C.

Il comportamento dello UES nella chirurgia oncologica della laringe

Atti Congresso Nazionale SIO Bologna 1983

8) Sartoris A., Giordano C., Di Fortunato V., Mioli P., Zara G.P., Potenzoni F.

Studio sperimentale sulla dinamica deglutitoria nei pazienti sottoposti ad

interventi conservativi della laringe

Rivista Italiana di Otorinolaringologia Audiologia e Foniatria 1985, 5:99-104

9) Zara G.P., Mioli P., Gaglia P., Eandi M.

Azione tachifilattica della pentagastrina sullo sfintere basso esofageo

In: Manometria e pHmetria esofagea. ed. F. Mattioli. Piccin Editore pp. 107-108

10) Zara G.P., Gaglia P., Mioli P., Eandi M.

The gastrin receptor of human LES.

Abstract 7th International Congress of Pharmacology Paris 1977

11) Mioli P., Zara G.P., Gaglia P., Caramalli S.

Ruolo della manometria esofagea nella diagnostica della neuropatia diabetica autonoma

In: Manometria e pHmetria esofagea nella diagnostica della neuropatia

diabetica autonoma. ed. F. Mattioli. Piccin Editore Padova

12) Farina E.C., Caramia M., Manzone E., Pinna Pintor M., Zara G.P., Mioli P.,

Belforte G., D'Alfio N.

The fluid-filled system for recording of the endoluminal pressures: compared study

Nota tecnica n. 131 dell'Istituto di Meccanica Applicata alle Macchine, Aerodinamica e Gasdinamica del Politecnico di Torino. Febbraio 1981

13) Zara G.P., Caramia M., Farina E.C., Manzone E., Pinna Pintor M., Mioli P., Belforte G., D'Alfio N.

The dynamic characteristic of the fluid-filled system for the recording of the endoluminal pressures of the gastrointestinal tract

Nota tecnica 132 dell'Istituto di Dinamica Applicata alle Macchine, Aerodinamica e Gasdinamica del Politecnico di Torino. Febbraio 1981

14) Vitelli A., Caramalli S., Carone R., Gaglia P., Mioli P., Peracchini M., Ressa G., Zara G.P.

Sensibilit  di alcune metodiche strumentali per il rilevamento delle alterazioni funzionali del sistema nervoso autonomo in corso di diabete mellito

Minerva Endocrinologica 1979, 4:111-116

15) Gaglia P., Pinna Pintor M., Zara G.P., Mioli P.

Effetti a distanza della resezione gastrica sullo sfintere esofageo inferiore

Minerva Chirurgica 1978, 33:1-5

16) Gaglia P., Mioli P., Zara G.P.

Lo sfintere esofageo inferiore nel gastrosigomiotomia studio manometrico

Minerva Chirurgica 1978, 33:1059-1066

17) Zara G.P., Mioli P., Farina E.C., Manzone E., Pinna Pintor M.

Action of TRH infusion on the human LES

Abstract :International Symposium on Brain-Gut axis: new frontiers. Florence 1981

18) Zara G.P., Mioli P., Pinna Pintor M., Balistreri F., Cassolino P.

Studio della motilita' intestinale in soggetto affetto da pseudo ostruzione intestinale cronica

Abstract: Giornate gastroenterologiche italo-britanniche. Capri 1985

19) Mioli P., Conte M., Todros L., Balistreri F., Zara G.P., Pinna Pintor M.

Dolore retrosternale atipico: malattia coronarica o esofagea

Abstract: Congresso congiunto SIPAD e AICED . Padova 1985

20) Kumar D., Browning C., Wingate D.L., Zara G.P., Quin X.Y., Johnson S.

Motility on the move.

Gastroenterology 1986, (Abstract) 90:1505

21) Mioli P.R., Caramia M., Farina E., Gaglia P., Manzone E., Pinna Pintor M.,

Zara G.P., Masenti E.

Valutazione della indicazione al trattamento chirurgico dell'ernia iatale.

Atti della Societa' italiana di chirurgia 1979, Vol.II. Comunicazioni.

22) Pinna Pintor M., Gaglia P., Manzone E., Mioli P., Zara G.P. Cosiderazioni anatomo cliniche su alcuni casi di tumore benigno dello stomaco.

Bolletino della societa' Piemontese di Chirurgia 1979

23) Conte M.R., Orzan F., Magnacca M., Brusca A., Zara G.P., Mioli P., Todros L.

Atypical chest pain: coronary or esophageal disease?

International Journal of Cardiology 1986, 13:135-142

24) Zara G.P., Thompson H.H.

Gastrointestinal effects of an alpha-2 adrenergic agent.

Gastroenterology 1987, 92:1708

25) Zara G.P., Balistreri F., Cassolino P.

La motilita' gastrointestinale. Alterazioni pre e post chirurgiche.

Atti del Corso di aggiornamento PROCTOLOGIA OGGI . Torino 15-16 Ottobre 1985.

26) Hutton M., Pilot M.A., Primi M.P., Thompson H.H., Zara G.P.
Effects of alpha-2 agents and capsaicin on intestinal transit after laparotomy.
J. Physiology 1988, 396:151P

27) Hutton M., Pilot M.A., Primi M.P., Thompson H.H., Zara G.P.
Effect of capsaicin on the nociceptive reflex during laparotomy in rat.
Regulatory Peptide 1987, 18:355

28) G.P. Zara, X.Y. Quin, Pilot M.A., Thompson H.H., Maskell G.
Erythromycin and gastrointestinal motility.
The Lancet October 31 1987: 1036

29) P. Mioli, F. Balistreri, P. Cassolino, M. Pinna Pintor, G.P. Zara, E. Masenti, M. Dei Poli.
Le conseguenze anatomo-funzionali della chirurgia conservativa dell'apparato digerente.

Nutrizione e cancro Torino 4-5 Dicembre 1987 pp.48-62

30) G.P. Zara, T.G. Barnett, D.R. Meeks, M.A. Pilot, H.H. Thompson.
Un registratore per lo studio prolungato della motilita' gastrointestinale nell'uomo e nel cane.
Atti del Congresso GISMAD. Cortina 29-30 Gennaio 1988

31) G.P. Zara, M.A. Pilot, H.H. Thompson.
Alterazioni della motilita' gastrointestinale indotte da eritromicina.
Atti del Congresso GISMAD. Cortina 29-30 Gennaio 1988

32) M.P. Primi, G.P. Zara, M.A. Pilot.
Effetto centrale e periferico della serotonina sulla motilita' intestinale del ratto.
Atti del Congresso GISMAD. Cortina 29-30 Gennaio 1988

- 33) G.P. Zara, M. Hutton, M.P. Primi, M.Pilot, H.H. Thompson
Effetto della capsaicina e dei bloccanti alfa-2 adrenergici sul transito intestinale
e dopo laparatomia nel ratto.
Atti del Congresso GISMAD. Cortina 29-30 Gennaio 1988.
- 34) G.P. Zara, X.Y. Quin, M.A. Pilot, H.H. Thompson.
Motor effects of erythromycin on the stomach and small intestine in man.
Gastroenterology 94:A-515 (1988)
- 35) G.P. Zara, M.A. Pilot, A. Bonabello H.H., Thompson, X.Y. Quin.
Erythromycin and canine colonic motility.
Gastroenterology 94:A-515 (1988)
- 36) M.R.E. Hutton, M.P. Primi, G.P. Zara, M.A. Pilot, H.H. Thompson.
Effects of capsaicin and alpha-2 adrenergic agents on intestinal motility after
laparotomy in rats.
Gastroenterology 94:A-198 (1988)
- 37) M.P. Primi, G.P. Zara, M.A. Pilot.
Central serotonergic control of gastrointestinal motility in rats.
Gastroenterology 94:A-359 (1988)
- 38) T.G. Barnett, M.A. Pilot, D.R. Meeks, H.H. Thompson, G.P. Zara.
A 4-channel solid-state recorder for GI motility studies an evaluation.
Gastroenterology 94:A-23 (1988)
- 39) H.H. Thompson, G.P. Zara, M.A. Pilot.
Motility changes following Roux-EN-Y gastrojejunostomy
Gastroenterology 94:A-459 (1988)
- 40) X.Y Quin, G.P. Zara, M.A. Pilot.

Effects of different macrolides on gastrointestinal motility

Abstract for the 8th International Symposium on Future Trends in
Chemotherapy pp. 169. Tirrenia 28-30 Marzo 1988

41) G.P. Zara, A. Bonabello, C. De Leo, T. Palumbo, A. Vannella, P. Mioli, E. Masenti.

A portable solid state logger for manometric and pH recording of the
gastrointestinal motility.

Abstract for the XXII Congress of the European Society for Surgical Research.
Bologna 2-5 Maggio 1988.

42) G.P. Zara, A. Bonabello, C. De Leo, T. Palumbo, P. Mioli, E. Masenti, G. Thomas.

Digital Recorder for gastrointestinal motility.

Abstract for XIII International Congress of Gastroenterology Rome, September
4-10 1988. Gastroenterology International 1988,1(1):749.

43) G.P. Zara, A. Bonabello, C. De Leo, T. Palumbo, P. Mioli, E. Masenti, G. Thomas.

A portable solid state recorder for manometric and Ph recording of the
esophageal motility.

Abstract for the 4th European Symposium on Gastrointestinal motility. Krakow
22-24 September 1988.

44) M.R.E. Hutton, M.A. Pilot, M.P. Primi, G.P. Zara

Effects of capsaicin and alpha-2 adrenergic agents on intestinal motility after
laparotomy in the rat.

Abstract for the 4th European Symposium on Gastrointestinal Motility. Krakow
22-24 September 1988. Hepato-gastroenterology 1988, 35:189.

45) T.G. Barnett, T.H. Koeze, M.A. Pilot, X.Y. Quin, H.H. Thompson, G.P. Zara.

The dataman: a solid state recorder for ambulatory and bench

recordings in dogs and humans.

Abstract for the 4th European Symposium on Gastrointestinal Motility. Krakow
22-24 September 1988. pp.109

46) M.P. Primi, M.A. Pilot, G.P. Zara.

A central action for serotonin in the control of gastrointestinal motility in fed rats.
Abstract for the 4th European Symposium on Gastrointestinal Motility. Krakow
22-24 September 1988. Hepato-gastroenterology 1988, 35:193.

47) X.Y. Quin, M. Scott, M.A. Pilot, H.H. Thompson, G.P. Zara, A. Bonabello.
Canine colonic motility changes after erythromycin.

Abstract for the 4th European Symposium on Gastrointestinal Motility. Krakow
22-24 September 1988. Hepato-gastroenterology 1988, 35:209.

48) G.P. Zara, A. Bonabello, C. De Leo, T. Palumbo, G. Thomas, C. Arduino,
M. Eandi.

Solid state logger for clinical pharmacological studies of the gastrointestinal
motility.

Pharmacological Research Communications 1988, 20(Suppl. 2):401.

49) F. Andreoli, S. Ricci Gamalero, C. Arduino, G.P. Zara, M.Eandi.

Hippocampal imipramine binbing and behavioural depression model in rat.

Pharmacological Research Communications 1988, 20(Suppl. 2):7

50) C. Arduino, G.P. Zara, F. Andreoli, S. Ricci Gamalero, M. Eandi.

Gastrointestinal transit and nifedipine oral bioavailability in rat.

Pharmacological Research Communications 1988, 20(Suppl. 2):9

51) G.P. Zara, M.A. Pilot, M. Hutton, C. Arduino and M. Eandi.

Visceral afferents of gastrointestinal nociceptive stimuli to the spinal cord.

Pharmacologica Research Communications 1988, 20(Suppl. 1):203.

52) A. Bonabello, G.P. Zara, C. De Leo, T. Palumbo, G. Thomas, P. Mioli, E. Masenti.

Registrazione ed analisi digitale della motilita' gastrointestinale nell'uomo e nel cane.

Abstract for Attualita' e prospettive dell'informatica in medicina. Firenze 26-29 settembre 1988.

53) G.P. Zara, A. Bonabello, C. De Leo, T. Palumbo, P. Mioli, F. Paties, G. Thomas, E. Masenti.

Portable digital recorder for the gastrointestinal motility
Gastroenterology 96:A564 1989.

54) P. Mioli, P. Cassolino, M. Baccega, F. Balistreri, M. Dei Poli, G.P. Zara, E. Masenti.

Clinical evaluation of the patients after oesophago-gastro-plasty for oesophageal carcinoma.

Gastroenterology 96:A346 1989

55) G.P. Zara, A. Bonabello, M. Eandi.

Pharmacokinetics of Ca⁺⁺ entry bloker and GI motility

International Symposium Dose-Response-Relationship of Drugs
Wuppertal 28-30 July 1989.

56) A. Bonabello, G.P. Zara, F. Paties, G. Thomas, M. Eandi.

Kinetics of the effects of Ca⁺⁺ entry bloker nifedipine on the cardiovascular and gastrointestinal system in conscious dogs.

In: Dose-Response Relationship of drugs. ed. by J. Kulmann, W. Wingender. W. Zuckschwerdt Verlag Munchen. pp. 140-143 1991.

57) A. Bonabello, G.P. Zara, C. De Leo, T. Palumbo, G. Thomas, P. Mioli, E. Masenti

Digital recording and analysis of gastrointestinal electromechanical activity.

Proceedings of Medinfo 89. Singapore 10-14 December 1989.
North-Holland New York 1989. pp.622-625

58) G.P. Zara, A. Bonabello, C. De Leo, P. Mioli, E. Masenti. Registrazione digitale dell'attivita' elettromeccanica intestinale. Atti della Societa' Italiana di Gastroenterologia Padova 13-16 Ottobre 1989.

59) G.P. Zara, A. Bonabello, F. Paties, P. Mioli, G. Thomas, M. Eandi.
Kinetics of the effects of nifedipine on the gastrointestinal and cardiovascular system.
Gastroenterology 98: A402 1990

60) M. Pinna Pintor, G.P. Zara, E. Falletto, A. Maraschiello, L. Monge, M. Dematteis.
Pelvic floor neuropathy in diabetic patients with faecal incontinence.
J. of Gastrointestinal Motility 1990 2:156

61) M. Pinna Pintor, G.P. Zara, E. Falletto, A. Maraschiello, L. Monge, M. Dematteis.
Pelvic floor neuropathy in diabetic patients with faecal incontinence.
Gastroenterology 98: A381 1990

62) A. Bonabello, G.P. Zara, F. Paties, P. Mioli, G. Thomas, M. Eandi.
Cinetica degli effetti della nifedipina sui sistemi gastrointestinali e cardiovascolare.
Proceedings IV Congresso Nazionale GISMAD pp. 14 1990

63) E. Falletto, L. Monge, V. Contegiacomo, G.P. Zara, M. Pinna Pintor.
Neuropatia del pavimento pelvico in pazienti diabetici con incontinenza fecale.
Proceedings IV Congresso Nazionale GISMAD pp. 35

64) P. Mioli, P. Cassolino, M. Pinna Pintor, G.P. Zara

I disturbi della motilità esofago-gastroduodenale indotti dalla chirurgia nelle esofagogastroplastiche.

Proceedings IV Congresso Nazionale GISMAD pp.61 1990

65) G.P. Zara, A. Bonabello, F. Signorile, C. Di Leo, P. Mioli
Registratore digitale dell'attività elettromeccanica dell'intestino.
Proceedings IV Congresso Nazionale GISMAD pp. 92 1990

66) G.P. Zara, X.Y. Quin, M.A. Pilot, H. Thompson, J. Maskell
Response of the Human Gastrointestinal Tract to erythromycin
J.Gastrointestinal Motility 1991, 3:26-31

67) M. Pinna Pintor, G.P. Zara, E. Falletto, L. Monge, M. Dematteis, Q. Carta, E. Masenti
Pudendal neuropathy in diabetic patients with faecal incontinence
Int. J. Colorectal Disease 1994, 9: 105-109

68) M.F. Di Renzo, R.P. Narsimhan, M. Prat, M. Olivero, S. Bretti, T. Crepaldi, P. Gaglia, G.P. Zara, P.G. Natali, P.M. Comoglio.
Expression of the c-MET/HGF receptor gene in human tumors
Proceedings of the European Workshop Genetic Alteration in Human Solid Tumors. Montpellier May 9-11 1991

69) M.F. Di Renzo, R.P. Narsimhan, M. Prat, M. Olivero, S. Bretti, T. Crepaldi, P. Gaglia, G.P. Zara, P.G. Natali, P.M. Comoglio.
Expression of the MET/HGF receptor gene in human tumors
Proceedings of the Second Meeting on The Molecular Basis of Human Cancer. Maryland U.S.A 19-22 June 1991.

70) D. Becchio, A. Cavicchioli, M.E. Magnino, I. Berra, G.P. Zara, G. Narduzzo, M. Eandi
Multimedia and hypertext programs for nursing education.

Proceedings of MIE 91 Vienna 19-22 Agosto 1991.D.

71) D.Becchio, G.P. Zara, M. Eandi, L. Olivero

Hypertext and multimedia programs for medical and nursing education.

Medicina Informatica submitted

72) D. Becchio, G.P. Zara

I sistemi ipertestuali e multimediali per la formazione e la ricerca infermieristica.

Atti del Breve Corso di Informatica Infermieristica Torino 27/2-1/3 1991

73) G.P. Zara, P. Mioli, P. Cassolino, V. Debernardi, A. Bonabello.

La Manometria digitale ambulante

Minerva Chirurgica 1991, vol. 46 Suppl.:61-62

74) G.P. Zara, A. Bonabello, C. De Leo, T. Palumbo, P. Mioli, E. Masenti, G.

Thomas

La registrazione della motilità gastrointestinale ambulante digitalizzata.

Atti Congresso Società Italiana di Chirurgia Roma 23-27 Ottobre 1988, pp. 419.

75) P. Mioli, M. Pinna Pintor, G.P. Zara, F. Trombetta, F. Balistreri, P.

Cassolino.

Caso di pseudoostruzione intestinale cronica (CIP), su base miopatica degenerativa.

Atti Secondo Congresso GISMAD Genova 7-8 Marzo 1986 pp. 95

76) P. Mioli, M. Pinna Pintor, G.P. Zara, P. Cassolino, F. Trombetta, F.

Balistreri.

Studio manometrico della motilità gastrointestinale insoggetti affetti da grave stipsi.

Atti Secondo Congresso GISMAD Genova 7-8 Marzo 1986 pp.116

77) A. Sartoris, C. Giordano, V. Di Fortunato, G. Succo, P. Mioli, G.P. Zara, F. Potenzoni.

Fisiopatologia della dinamica deglutitiva nei pazienti sottoposti ad interventi conservativi e demolitivi della laringe (studio sperimentale).

Il Giornale di Chirurgia 1986, 7:1505-1507

78) M. Garavoglia, P. Mioli, E. D'Ambrosio, E.C. Farina, E. Manzone, M. Pinna Pintor, G.P. Zara.

La diagnosi endoscopica nella patologia funzionale del giunto gastro-esofageo.

Atti Simposio Nazionale di Endoscopia Digestiva Napoli 14-16 Novembre 1980.
pp.44

79) P. Mioli, P. Dusio, M. Pinna Pintor, P. Puiatti, G. P. Zara

Alterazioni della motilità esofagea in pazienti sclerodermici.

Atti Primo Congresso GISMAD Bologna 4-5 Novembre 1983 pp. 22

80) P. Mioli, P. Dusio, E. Manzone, M. Pinna Pintor, G.P. Zara, M.R. Conte, M. Magnacca.

Ruolo della patologia esofagea nel dolore toracico simil-anginoso.

Atti Primo Congresso GISMAD Bologna 4-5 Novembre 1983 pp. 22

81) P. Mioli, M. Pinna Pintor, P. Dusio, G.P. Zara, T.E. Adrian, S.R. Bloom.

Effects of TRH infusion on the Lower Esophageal Spincter in Man.

Proceedings of the International Meeting on Gastrointestinal motility in health and disease. Madrid May 12-13 1983.

82) M. Eandi, G.P. Zara, S. Ricci Gamalero, P. Mioli, P. Taglia, P. Gaglia

Tachyphylactic action of high doses of pentagastrin

Boll.Soc.It.Biol.Sper. 1979, vol.LV:2145-1251

83) M.F. Di Renzo, R.P. Narsimhan, M. Olivero, S. Bretti, Giordano S., Medico

E., P. Gaglia, G.P. Zara, P.G. Natali, P.M. Comoglio.

Expression of the Met/HGF receptor in normal and neoplastic human tissues.
Oncogenes in press

84) G.P. Zara, Adrian T.E., Mioli P., Pera A., Manzone E., Dusio P., Bloom S.R.
TRH action on the lower esophageal sphincter (LES) in man
Abstract Fourth International Symposium on Gastrointestinal Hormones
Stockholm 1982

85) D. Becchio, G. P. Zara, M. Eandi, P. Lupano, G. Lenta
Computer-based training with multimedia and hypertext technology
Proceedings British health Computer Society Harroghate March 1992.
pp. 107-112

86) L. Monge, M. Pinna Pintor, E. Falletto, G.P. Zara, M. De Matteis,
Q. Carta
Pelvic neuropathy in diabetic patients with faecal incontinence.
Diabetes 1991, 40(Suppl.1): 510A.

87) Maria Enrica Magnino, G.P. Zara
Infermieristica ed applicazioni Informatiche
Proceedings Congresso Nazionale Hospital 92. Bologna 28/5/1992

88)G.P. Zara, C. Della Pepa, M. Eandi
Computer-Based-Learning in clinical pharmacology
Pharmacological Research 1992, 26:279

89)M. Costa, C. Della Pepa, G.P. Zara, M. Eandi
Artificial Neural Network for Pharmacokinetics modellin
in: Measurement and Kinetics of in vivo drug effect
1994, 104-105

90) C. Della Pepa, M. Costa, G.P. Zara, M. Eandi

Artificial Neural Networks (ANNs) applied to the Pharmacokinetic
pharmacodynamic (PK/PD) studies
Abstract XXVII Congresso Nazionale della Societa' Italiana di Farmacologia
1994, 65

91) G.P. Zara, C. Della Pepa, M. Eandi
Hypertext and multimedia programs for teaching pharmacology
Abstract XXVII Congresso Nazionale della Societa' Italiana di Farmacologia
1994, 116

92) G.P. Zara, C. Della Pepa, M. Eandi
Hormonal control of gastrointestinal motility
Abstract XXVII Congresso Nazionale della Societa' Italiana di Farmacologia
1994, 661

93) M. Eandi, C. Della Pepa, G.P. Zara, Silvana Ricci Gamalero
Le Tossicodipendenza ed i comportamenti d'abuso
In: Aids e Tossicodipendenza, CESPI Editore 1994, 13-29

94) C. Della Pepa, M. Eandi, G.P. Zara, Luisa Taricco
Le sostanze d'abuso
In: Aids e Tossicodipendenza, CESPI Editore 1994, 31-54

95) G.P. Zara, C. Della Pepa, I. Carbone, A. Rava, S. Ricci Gamalero, M. Eandi
Computer based multimedia for teaching clinical pharmacology
Can. J. physiol. Pharmacol 72 (Suppl. 1): 288

96) A. Rava, G.P. Zara, C. Della Pepa, I. Carbone, M. Eandi
Clinical trial activities tutor (CTAT): an interactive computer program to help
investigators to comply with GCPs
Can. J. physiol. Pharmacol 72 (Suppl. 1): 288

97) C. Della Pepa, G.P. Zara, A. Rava, I. Carbone, M. Eandi, S. Ricci
Gamalero, A. Ottobrelli, M. Lagget, G.C. Actis
Population pharmacokinetic of cyclosporine in patients with ulcer colitis
Can. J. physiol. Pharmacol 72 (Suppl. 1): 301

98) S. Ricci Gamalero, C. Zaccaro, G.P. Zara, C. Della Pepa
A New behavioral test for early detection of drug neurotoxicity
Can. J. physiol. Pharmacol 72 (Suppl. 1): 420

99) N. Dirindin, G.P. Zara
Strumenti di valutazione economica del farmaco
Farmacoeconomia Giugno 1994 : 5-10 1994

100) G.P. Zara
Rapporto costo-efficacia del trattamento precoce con AZT in soggetti HIV-positivi
Farmacoeconomia Giugno 1994 : 22-23 1994

101) G.P. Zara
Razionalizzazione dei farmaci in ospedale: assegnazione delle priorit 
Farmacoeconomia Settembre 1994 : 26-29 1994

102) G.P. Zara
Bibliografia cronologica mondiale sul tema "Prontuario ospedaliero"
Farmacoeconomia Giugno 1994 : 36-50 1994

103) G.P. Zara
Per saperne di piu' su QoL e chemioterapia
Farmacoeconomia Marzo 1995 : 25-31 1995

104) G.P. Zara

L' utilizzo dell' unita' di misura DDD nella ricerca epidemiologica sull' utilizzo dei farmaci

Farmacoeconomia Settembre 1995 : 25-30 1995

105) Eandi M., Zara G.P., Della Pepa C., Dirindin N.:

Farmacoeconomia: un nuovo approccio alle strategie terapeutiche. In Antibiotica Update (Congress Report) - IX Congresso Mediterraneo di Chemioterapia, Milano, 12-17, 25-31, Giugno 1994.

106) C. Della Pepa, G.P. Zara

Disordini Gastrointestinali da Farmaci

in: Argomenti di Gastroenterologia ed. by A. Pera et al. , Archimedita Editori Torino. pp:91-104, 1994

107) G.P. Zara, I. Carbone, M. Eandi

Internet per la formazione e l' informazione medica

III Seminario di Informatica in Nefrologia

Torino 9-10 marzo 1995

108) G.P. Zara, I. Carbone, M. Eandi

I sistemi multimediali nella didattica medica

III Seminario di Informatica in Nefrologia

Torino 9-10 marzo 1995

109) G.P. Zara

Aggiornamento in tema di "costo di malattia"

Farmacoeconomia Marzo 1996 , 40-45

110) A. Rava, M. Eandi, G.P. Zara, N. Dirindin, F. Vanara, P. Pederzoli, M.

falconi, C. Bassi

Incremental cost-effectiveness analysis of gabexate mesilate versus aprotinin in human acute pancreatitis

Acta Physiologica Pharmacologica Therapeutica Latinoamericana 46 (6): 252,
1996

111) M. Eandi, M. Costa, C. Della Pepa, R. Canaparo, G.P. Zara,
Antibiotic dosage individualization through bayesian inference on PK-PD
population data
Acta Physiologica Pharmacologica Therapeutica Latinoamericana 46 (6): 236,
1996

112) G.P. Zara, I. Carbone, E. Muntoni, M. Eandi
MEDFARM: an Internet based resource to distribute clinical pharmacology
information
Acta Physiologica Pharmacologica Therapeutica Latinoamericana 46 (6): 267,
1996

113) D. Tartaro, D. Becchio, G.P. Zara, N. Dirindin
Il costo dei materiali d'uso e remunerabilita' delle diverse linee di prodotto
Farmacoeconomia Settembre 1996 : 5-11 1996

114) M.R. Gasco, A. Bargoni, R. Cavalli, E. Conti, A. Fundaro', G.P. Zara
Transport in Lymph and blood of solid nanoparticles after oral administration in
rats
Proceedings of the 24th International Symposium on Controlled Release of
Bioactive Materials 1997, 179-180

115) M. Eandi, M. Costa, G.P. Zara, R. Canaparo, C. Della Pepa, E. Muntoni
Individualization of Ceftriaxone dosage through Bayesian Inference on PK-PD
population data
Proceedings of the 20th International Congress on Chemotherapy 1997, 21

116) G. Fornasini, N. Monti, G. Brogini, M. Gallina, M. Eandi, S. Persiani, M.
Bani, C. Della Pepa, G.P. Zara, M. Strolin Benedetti

Preliminary Pharmacokinetic Study of Ibuprofen enantiomers after administration of a new oral formulation (Ibuprofen Arginine) to healthy male volunteers

Chirality 1997, 9:297-302

117) S. Ricci-Gamalerò, C. Zaccaro, R. Canaparo, G.P. Zara, A. Fundaro' and M. Eandi

A New Behavioural Test for early detection of drug teratogenicity and peri-post natal neurotoxicity in rats.

Pharmacological Research 35, 3:225 1997

118) M. Costa, C. Della Pepa, G.P. Zara, M. Eandi

A non-parametric predictive approach of individual PK/PD quantities from sparse clinical data

Pharmacological Research 35, 3:230 1997

119) M. Eandi, M. Cosat, A. Costa Laila, C. della Pepa, N. Dirindin, G.P. Zara

Bayesian autoclassification of general practitioner prescriptive style

Eur. J. Clin. Pharmacol. 53: A39 1997

120) A. Bargoni, R. Cavalli, Otto Caputi, A. Fundaro', M.R. Gasco, G.P. Zara

Solid lipid nanoparticles in lymph and plasma after duodenal administration in rats.

Pharmaceutical Research 15:745-750, 1998

121) M. Eandi, G. Recchia, G.P. Zara

Pharmacoeconomic: already in phase I?

Pharmacological Research 31(suppl.): 3 1995

122) M. Eandi, G.P. Zara

Economic Impact of resistance in community

International Journal of Clinical Practice Suppl. 91: 28-39, 1998

123) M. Costa, E. Berno, G.P. Zara, C. della Pepa, R. Canaparo, M. Eandi
A non-parametric population PK/PD analysis of Tolcapone to predict L-DOPA
plasma concentrations

Proceedings PAGE 98 Wuppertal 1998

124) R. Canaparo, M. Costa, E. Muntoni, C. Della Pepa, G.P. Zara, M. Eandi
Validation of a non-parametric approach to predict individual plasma
concentration from population data

Naunyn-Schmiedeberg's Archives of Pharmacology 385 (suppl 1):R469

125) C. Della Pepa, M. Costa, A. Costa Laila, L. Roccati, G.P. Zara, R.
Canaparo, M. Eandi

Prescriptive styles of general practitioner identified through Bayesian
autoclassification method

Naunyn-Schmiedeberg's Archives of Pharmacology 385 (suppl 1):R469

126) G.P. Zara, A. Fundaro, R. Cavalli, A. Bargoni, R. Corradino, O. Caputo,
M.R. Gasco, M. Eandi

Pharmacokinetics and Biodistribution of doxorubicin incorporated in solid lipid
nanoparticles

Naunyn-Schmiedeberg's Archives of Pharmacology 385 (suppl 1):R510

127) G.P. Zara, R. Cavalli, A. Fundaro, A. Bargoni, O. Caputo, M.R. Gasco
Pharmacokinetics of doxorubicin incorporated in solid lipid nanospheres (SLN)
Pharmacological Research, 40(3):281-286 1999

128) R.Canaparo, E. Muntoni, G.P. Zara, C. Della Pepa, E. Berno, M. Costa, M.
Eandi

Determination of Ibuprofen in human plasma by high performance liquid
chromatography: validation and application in pharmacokinetic study

Biomedical Chromatography 14:219-226, 2000

129) G.P. Zara, A. Fundaro, A. Bargoni, R. Corradino, R. Cavalli, M.R. Gasco, M. Eandi

Pharmacokinetics and Biodistribution of doxorubicin incorporated in solid lipid nanoparticles

Pharmacological Research 39 (suppl.): 12

130) R. Canaparo, N. Pons, F. Casale, E. Muntoni, G. Fornari, G.P. Zara, C. Della Pepa, M. Eandi

Plasma Immunoerythropoietin levels in patients with cancer receiving platinum-based or 5-Fluorouracil-based chemotherapy

Pharmacological Research 39 (suppl.): 12

131) R. Canaparo, N. Pons, F. Casale, E. Muntoni, G.P. Zara, C. Della Pepa, E. Berno, G. Fornari, M. Eandi

Plasma Erythropoietin levels in patients receiving intense platinum or non-platinum chemotherapy.

British Journal Clinical Pharmacology 50(2):146-153 2000

132) V. Podio, G.P. Zara, M. Carazzone, R. Cavalli, M. R. Gasco

Biodistribution of Solid Lipid Nanospheres after i.v. administration to Rats

J.Pharmacy and Pharmacology 52 (2000), 1057-1063

133) A. Bonabello, M.R. Galmozzi, T. Bruzzese, G.P. Zara

Analgesic Effect of Bisphosphonate in mice

Pain 2001, 91(3):269-275

134) G.P. Zara, M. Eandi

Pharmacoeconomics: Gastro-oesophageal reflux disease and irritable bowel syndrome. In Approach to the patient with chronic gastrointestinal disorders. Ed. by E. Corazziari. Messaggi 1999

135) R. Pellicano, F. Palmas, B. DeMarchi, G.P. Zara, M. Eandi

Farmaci e tratto digestivo superiore

Attualita' in Gastroenterologia - Minerva Medica 2000

136) R. Cavalli, G.P. Zara, O. Caputo, A. Bargoni, A. Fundaro, M.R. Gasco

Transmucosal Transport of Tobramycin incorporated in SLN after duodenal administration. Part I-Pharmacokinetic study

Pharmacol. Res. 42 (2000) 541-545

137) Fundarò A., Cavalli R., Bargoni A., Vighetto D., Zara G.P., Gasco M.R.,

Stealth and non-stealth solid lipid nanoparticles (SLN) carrying doxorubicin:

Pharmacokinetics and tissue distribution after I.V. administration to rats,

Pharmacol.Res 42 (2000) 337-343

138) Zara G.P., Bargoni A., Cavalli R., Fundarò A., Vighetto D., Gasco M.R.

Idarubicin Solid lipid nanospheres administration to rats by duodenal route:

Pharmacokinetics and Tissues distribution

J. Pharmaceutical Science 2002, 91 (5):1324-1333

139) A. Bargoni, R. Cavalli, G.P. Zara, A. Fundaro', O. Caputo, M.R. Gasco

Transmural transport of Tobramycin incorporated in SLN after duodenal administration. Part II. Tissue distribution

Pharmacol. Res. 43 (2001): 497-502

140) C. Della Pepa, A. Costa Laila, E. Berno, M. Costa, L. Rocatti, G.P. Zara,

R. Canaparo, M. Eandi

Costs Analysis and prescriptive styles of general practitioners identified by

Bayesian autoclassification method

British Journal Clinical Pharmacology Abstract CPT 2000: 98

141) R. Canaparo, E. Muntoni, L. Serpe, C. Della Pepa, G.P. Zara, F. Casale,

M. Eandi

HPLC determination of 5-FU and three metabolites in the plasma of cancer patients

British Journal Clinical Pharmacology Abstract CPT 2000: 105

142) C. Della Pepa, E. Muntoni, R. Canaparo, M. Visentin, G. Ferraris, E. Berno, G.P. Zara, M. Eandi

Variation of body weight and plasma lipid concentration on overweight patients treated with Citrus aurantium extracts and magnesium piruvate.

British Journal Clinical Pharmacology Abstract CPT 2000: 126

143) C. Della Pepa, E. Muntoni, R. Canaparo, P. Romano, F. Dosio, G.P. Zara, M. Eandi

Pharmacokinetics of disodium clodronate after single intravenous or intramuscular injection in health volunteers

British Journal Clinical Pharmacology Abstract CPT 2000: 206

144) M Eandi, G.P. Zara, C. Della Pepa

Application of pharmacoeconomics to the use of antibiotics.

Clinical Microbiology and Infection 6 Suppl.3(2000): 90-93

145) A. Bargoni, R. Cavalli, A. Fundaro', M.R. Gasco, D. Vighetto, G.P. Zara
Gastrointestinal absorption of idarubicin-loaded solid lipid nanoparticles.

Proceed.Int'l.Symp.Control.Rel.Bioact.Mat. Paris 2000: 1214-1215-2000

146) G.P. Zara, R. Cavalli, A. Bargoni, A. Fundaro', D. Vighetto, M.R. Gasco.
Intravenous administration to rabbits of non-stealth and stealth doxorubicin loaded solid lipid nanoparticles at increasing concentration of stealth agent: pharmacokinetics and distribution in brain and other tissues

Journal Drug Targeting 10 (2002): 327-335

147) G.P Zara., Cavalli R., Bargoni A., Fundaro' A., GascoM.R.

Gastrointestinal Absorbption of Tobramycin incorporated in solid lipid nanoparticles

Br. J. Pharmacol. 132 (2000): 251P, 2002

148) A. Bonabello, M.R. Galmozzi, T. Bruzzese, G.P. Zara

Analgesic effect of Biphosphonates in mice

Br. J. Pharmacol. 132 (2000): 164P, 2002

149) A. Bonabello, M.R. Galmozzi, T. Bruzzese, E. Muntoni, G.P. Zara

Analgesic, Anti-inflammatory and gastric-damaging effects of dexibuprofen in rodents.

Anesthesia & Analgesia 2003, 97: 402-408

150) G.P. Zara, A. Bargoni, R. Cavalli, M.R. Gasco, M.Eandi

Pharmacokinetic and biodistribution of idarubicin incorporated in solid lipid nanoparticles.

Pharmacology and Toxicology 2001, 89 (Suppl. 1): 92

151) M.R. Galmozzi, G. Buffa, C. Rossi, T. Bruzzese, G.P. Zara, A. Bonabello

Clodronate has a long-lasting analgesic action

Pharmacology and Toxicology 2001, 89 (Suppl. 1): 134

152) M. Guido, A. Brioschi, A. Miglietta, R. Cavalli, C. Bocca, E. Peira, G.P.

Zara, M.R. Gasco, A. Mauro.

Cytotoxic effects of doxorubicin and paclitaxel incorporated in solid lipid nanoparticles against malignant glioma cells.

Neuro-Oncology 2001: 3(suppl. 1): S72

153) G.P. Zara, R. Cavalli, A. Bargoni, A. Mauro.

Brain targeting of anthracyclines incorporated in solid lipid nanoparticles.

Neuro-Oncology 2001: 3(suppl. 1): S82

154) R. Cavalli, A. Bargoni, V. Podio, E. Muntoni, G.P. Zara, M.R. Gasco
Solid Lipid nanoparticles loaded with different percentages of tobramycin in vitro
and in vivo kinetics after duodenal administration
J. Pharmac. Science 2003, 92 (5): 1085-1094

155) D. Vighetto, G.P. Zara, M.R. Gasco
Incorporation of flurbiprofen in solid lipid nanoparticles for trasdermal release.
J. Pharm. Sci. Submitted

156) L. Serpe, S. Laurora, S. Pizzimenti, G. Barrera, E. Ugazio, M.R. Gasco,
G.P. Zara, M. Eandi
Azione citotossica in vitro del colesterilbutirrato incorporato in nanoparticelle
lipidiche solide.
21 ° Congresso Nazionale della Societa' Italiana di Chemioterapia 2001: 188

157) F.Casale, R. Canaparo, E. Muntoni, L. Serpe, G.P. Zara, C. Della Pepa, E.
Berno, M. Costa and M. Eandi
Simultaneous HPLC determination of 5-fluorouracil and its metabolites in
plasma of cancer patients.
Biomedical Chromatography 2002 16(7):446-452

158) A.Bonabello, M. Galmozzi, T. Bruzzese, G.P. Zara
Analgesic effect of topical disodium clodronate in mice
Pharmacologist 2002, 44(2): A55

159) M. Eandi, C. Della Pepa, E. Muntoni, R. Canaparo, P. Romano, F. Casale,
L. Serpe, G.P. Zara
Single dose intramuscular pharmacokinetics and local tolerability of three
different disodium clodronate formulations in healthy volunteers.
Pharmacologist 2002, 44(2): A55

160) L Serpe, S Laurora, S Pizzimenti, E Ugazio, R Ponti, R Canaparo, F Briatore, G Barrera, MR Gasco, MG Bernengo, M Eandi, GP Zara
Cholesteryl butyrate solid lipid nanoparticles as a butyric acid pro-drug: effects on cell proliferation, cell cycle distribution and c-myc expression in human leukemic cells

Anti.Cancer Drugs 2004, 15:525-536

161) Bonabello, M.R. Galmozzi, T. Bruzzese, R. Canaparo, G.P. Zara
Long Term Analgesic Effect of Clodronate in rodents

BONE 2003, 33: 567-574

162) G.P. Zara, C. Della Pepa, L. Prandelli, M. Eandi
Osteoporosi: la malattia e il suo costo sanitario

Farmacoeconomia 2002, 3(4): 185-208

163) Muntoni, E., Canaparo, R., Della Pepa, C., Serpe, L., Casale, F., Barbera, S., Romano, P., Zara, G.P. and Eandi M

Determination of disodium clodronate in human plasma and urine by gas chromatography--nitrogen--phosphorus detection: validation and application in pharmacokinetic study.

Jnl. of Chromatography B - Anl.Tech. in the Biomed.& Life Sci 2004, 799:133-139

164) L Serpe, MG Catalano, R Cavalli, E Ugazio, O Bosco, R Canaparo, E Muntoni, R Frairia, MR Gasco, M Eandi, GP Zara

Cytotoxicity of anticancer drugs incorporated in solid lipid nanoparticles on HT-29 colorectal cancer cell line

Eur. J. Pharmaceutics Biopharmaceutics 2004, 58 (3): 673-680

165) Casale F, Canaparo R, Serpe L, Muntoni E, Della Pepa C, Costa M, Mairone L, Zara GP, Fornari G, Eandi M.

Plasma concentrations of 5-fluorouracil and its metabolites in colon cancer patients.

Pharmacol Res 50 (2004) 173–179

166) Federico Casale, Francesco Lupo, Roberto Passera, Guido Liddo, Donatella Moscato, Roberto Mosso, Gian Paolo Zara, Mario Eandi, and Mauro Salizzoni

Pharmacokinetics of cyclosporine microemulsion in recipients of marginal versus control liver transplants.

Pharmacol Res 2006, 54: 287-292

167) Dianzani C, Cavalli R, Zara GP, Gallicchio M, Lombardi G, Gasco MR, Panzanelli P, Fantozzi R.

Cholesterol butyrate solid lipid nanoparticles inhibit the adhesion of human neutrophils to endothelial cells

Br J Pharmacol 148:648-656 2006

168) Serpe L, Guido M, Canaparo R, Muntoni E, Cavalli R, Panzanelli P, Bargoni A, Gasco MR, Eandi M, Zara GP

Intracellular accumulation and cytotoxicity of Doxorubicin with different pharmaceutical formulations in human cancer cell lines.

J Nanosci Nanotechnol 2006, 6 (9-10):3062-3069

169) Zara GP, Cavalli R, Canaparo R, Serpe L, Muntoni E, Gasco MR, Eandi M
Ophthalmic delivery of dexamethasone and tobramycin incorporated in solid lipid nanoparticles.

Basic and Clinical Pharmacol & Toxicol 2005, 97:57

170) Serpe L, Cavalli R, Guido M, Canaparo R, Muntoni E, Gasco MR, Mauro A, Eandi M, Zara GP

Accumulation and cytotoxic activity of different doxorubicin formulations in human cancer cell lines

Basic and Clinical Pharmacol &Toxicol 2005, 97:57

171) Canaparo R, Serpe L, Calvo GL, Barbera C, Muntoni E, Eandi M, Zara GP
Phenotype and genotype between italian children and adults for thiopurine
methyltransferase

Basic and Clinical Pharmacol &Toxicol 2005, 97:121

172) Canapro R, Nordmark A, Finnstrom N, Lundgren S, Seidegard J,
Jeppsson B, Serpe L, Eandi M, Zara GP, Rane A.
CYP3A and MDR1 (P-glycoprotein) gene expression levels along the human
gastrointestinal tract.

Basic and Clinical Pharmacol &Toxicol 2005, 97:122

173) Canaparo R, Serpe L, Catalano, Bosco O, Zara GP, Berta O, Frairia R.
High Energy Shock Waves for sonodynamic therapy: in vitro effects on a human
colon cancer cell line (HT-29)

Anticancer Research 2006, 26:3337-3342

174) Cavalli R, Chetoni P, Tullio V, Gallicchio M, Dianzani C, Cuffini AM,
NajarroM, Bargoni A, Carlone NA , Fantozzi R, Gasco MR, Zara GP
Ophthalmic delivery in Rabbits of Dexamethasone and Tobramycin
incorporated in solid lipid nanoparticles

Invest Ophthalm Visual Sci submitted 2006

175) Ferruti P, Franchini J, Bencini M, Ranucci E, Cavalli R, Serpe L, Zara GP
A prevailingly cationic agmatine-based amphoteric polyamidoamine as non-
toxic, non-hemolytic and “stealthlike” DNA complexing agent and transfection
promoter

Biomacromolecules. 2007;8(5):1498-1504.

176) Battaglia L., Trotta M., Gallarate M., Parlotti ME, Zara GP, Bargoni A.

Solid Lipid nanoparticles formed by solvent-in-water emulsion-diffusion
technique: Development and influence on insulin stability
Journal of Microencapsulation 2007, 34: 1-13

177) Serpe L, Canaparo R, Daperno M, eabdi M, Gasco M, Zara GP
In vitro effects of anti-inflammatory drugs incorporated in solid lipid
nanoparticles on human peripheral mononuclear blood cells from IBD patients
Basic & Clinical Pharmacology & Toxicology 2007, 101:71
178)Zara GP, Cattaldo S, Cavalli R, Calderoni S, Muntoni E, Gasco MR, Mauro
M, Eandi M

A new apomorphine formulation for oral administration
Basic & Clinical Pharmacology & Toxicology 2007, 101:103

179) Canaparo R, Serpe L, Eandi M, Zara GP, Berta L, Frairia R
High Energy Shock waves increase paclitaxel efficacy in a syngenic model of
breast cancer
Basic & Clinical Pharmacology & Toxicology 2007, 101:118

180) Canaparo R, Finnström N, Serpe L, Nordmark A, Muntoni E, Eandi M,
Rane A, Zara GP.
Expression of CYP3A isoforms and P-glycoprotein in human stomach, jejunum
and ileum.
Clin Exp Pharmacol Physiol. 2007, 34(11):1138-44

181) Brioschi A, Zara GP, Calderoni S. Gasco MR, Mauro A.
Cholesterylbutyrate Solid Lipid Nanoparticles as Prodrug of Butyric Acid
Molecules 2008, 13:230-254

182) Brioschi A., Zenga F., Zara G.P., Gasco M.R., Ducati A., Mauro A.
Solid lipid nanoparticles: Could they help to improve the efficacy of
pharmacologic treatments for brain tumors? Neurological Research. 2007,
29(3): 324-330.

183) G.P. Zara

Tossicologia

In Farmacologia Generale e Applicata. M.Memo, F. DePonti, M. Eandi ed.
Monduzzi 2008, 207-228

184) Brioschi A, Calderoni S, Pradotto LG, Guido M, Strada A, Zenga F,
Benech CA, Benech F, Serpe L, Zara GP, Musicanti C, Ducati A, Gasco MR,
Mauro A. Solid Lipid Nanoparticles carrying oligonucleotides inhibit Vascular
Endothelial Growth Factor expression in rat glioma models.
J Nanoneurosci 2008; 1:1-10